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Synthesis of polysaccharides having specific biological activities

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Abstract

Several types of stereoregular polysaccharides such as linear, branched, amino, and deoxy polysaccharides were synthesized by ring-opening polymerization of anhydro-sugar derivatives to reveal the relationship between structure and biological activities. Their stereoregular and naturally occurring polysaccharides were sulfonated. It was found that the sulfonated polysaccharides had potent anti-human immunodeficiency virus (anti-HIV) activities, which increased with an increase of the proportion of amino sugar and branched units on the main chain, but with a decrease of deoxy sugar units and branches. Another biological activity of sulfonated polysaccharides, blood anti-coagulant activity, was also examined. An overview will be presented of various synthetic and natural polysaccharides having biological activities.

Keywords: Anhydro-sugars; Anhydro-disaccharides; Amino sugars; Ring-opening polymerization; Enzymatic polymerization; Branched polysaccharides; Amino polysaccharides; Deoxy polysaccharides; Sulfonated polysaccharides; Anti-HIV activity; Blood anti-coagulant activity; Anti-tumor activity; Wound healing activity

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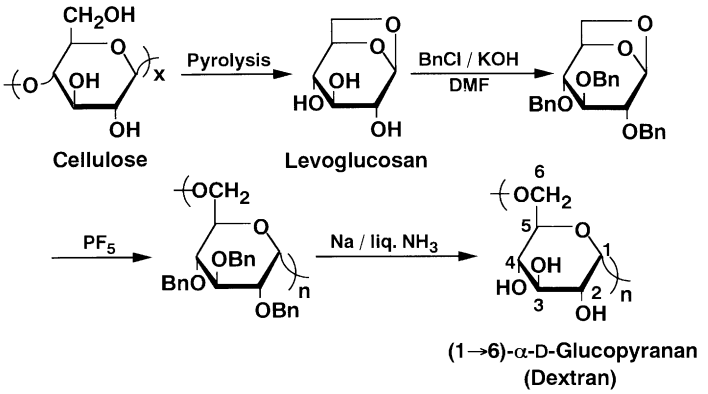
1. Introduction

Much attention has been paid to key roles played in a wide range of biological recognitions of naturally occurring poly- and oligosaccharides in proteoglycans, glycoproteins, and glycolipids on the surface of cells. Since, in general, natural polysaccharides have complex structures due to their heterogeneity including many kinds of monosaccharide units, it is difficult to elucidate the relationship between structures and activity, even though new instrumental analyses have been developed now. In order to know the relationship and then to synthesize the new materials having specific biological activities, the synthesis of polysaccharides having defined structures becomes an important technology.

The ring-opening polymerization is an excellent method for providing stereoregular polysaccharides having high molecular weights. Appropriate combinations of hydroxyl-protective groups in anhydro-sugars, catalysts, and polymerization temperatures obtain polysaccharides with the desired stereoregularity. Recently, some reports appeared on the synthesis of cellulose or cello-oligosaccharides by the stepwise coupling reaction of cello-oligosaccharide, orthoester condensation of D-glucose derivative, and enzymatic polymerization. Unfortunately, cellulose has not been synthesized by the ring-opening polymerization of anhydro-sugars.

Anhydro-sugars are bicyclic monosaccharides having 1,6- and 1,5-anhydro rings for hexoses and 1,4- and 1,5-rings for pentoses, respectively, which are prepared by a conventionally reduced pyrolysis of the corresponding monosaccharides or polysaccharides such as cellulose, amylose, and mannan. Quite recently, it was reported that the starting anhydro-sugars were obtained easily and directly by the microwave-rapid pyrolysis of lumbers. Although Pictet first reported the synthesis and ring-opening polymerization of an anhydro-sugar such as 1,6-anhydro-α-D-glucopyranose (levoglucosan) in 1918 [1,2], no stereoregular polysaccharides were obtained. In 1966, Ruckel and Schuerch reported for the first time the synthesis of stereoregular (1 → 6)-α-D-glucopyranan (dextran) by the ring-opening polymerization of benzylated levoglucosan [3] (Scheme 1).



Scheme 1.

In 1987, Nakashima and Yamamoto found the anti-human immunodeficiency virus (anti-HIV) activity of natural sulfonated polysaccharides from sea alga [4,5] and we also revealed the anti-HIV activity of synthetic sulfonated polysaccharides obtained by the ring-opening polymerization of anhydro-sugars [6].

The first purpose of this review is to reveal the recent advances in the synthesis of polysaccharides having defined structures. The second purpose is to account for the structure–biological activity relationship of the synthetic polysaccharides. Some biological results on natural polysaccharides are also included. Results before 1993 will not be discussed so much because Uryu reviewed artificial polysaccharides and their biological activities in this journal in 1993 [7], and other excellent reviews and publications appeared already [8–12].

2. Synthesis of linear polysaccharides

2.1. Recent synthesis of anhydro-sugar monomers

2.1.1. 1,6-Anhydro-α-D-glucopyranose (levoglucosan)

In general, anhydro-sugars were obtained by the vacuum pyrolysis of plant products such as cellulose, ivory nuts, and monosaccharides. The first finding of anhydro-sugar, 1,6-anhydro-β-D-glucopyranose (levoglucosan), by pyrolysis of cellulose, was reported by Pictet and Sarasin in 1918 [1]. It was reported in 1966 that levoglucosan was obtained in high yield (44%) when cellulose was pyrolyzed at 456°C for 6 min [13]. However, amounts of the starting cellulose were small (1–2 g). The purpose of pyrolysis in these periods was to elucidate the thermal decompositions of finding new flame-retardant treatments.

For the starting materials of the ring-opening polymerization, the vacuum pyrolysis was carried out by using large amounts of sugars because the yield of anhydro-sugars was not so high, 5–10%. For example, microcrystalline cellulose (400 g × 5 times) was pyrolyzed by the conventional apparatus as shown in Fig. 1 under vacuum to give about 200 g (10% yield) of levoglucosan. When other monosaccharides such as D-ribose, D-xylose, and L-arabinose were pyrolyzed under similar conditions, the corresponding 1,4-anhydro-α-D-ribopyranose, -D-xylopyranose, and -L-arabinopyranose were obtained at around 10% yields.

In recent years, microwave-assisted reactions have been applied to organic synthesis [14,15]. In 1980, it was first reported that the microwave irradiation to a cellulose pulp pellet from ash-free analytical filter pulp (2.6 g) gave levoglucosan in 27% yield [16]. In 1988, levoglucosan was obtained by the microwave irradiation of starch or other 1,4-glucans in a conventional microwave oven in 1% yield [17]. With the laboratory-scale microwave reactor, pyrolysis of cellulose in dilute sulfuric acid afforded glucose in 39% yield [18]. However, there was no information on the weights of cellulose used.

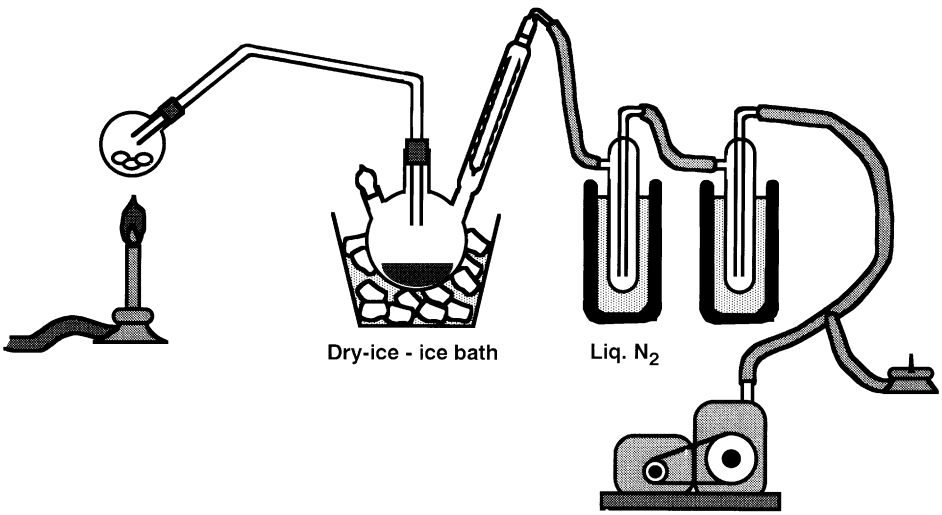
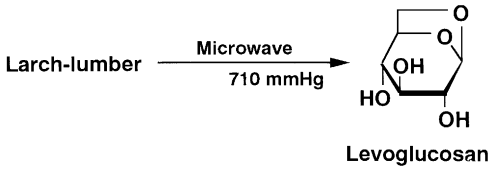


Fig. 1. Thermal pyrolysis apparatus by gas heating.



Scheme 2.

In 1991, Miura developed a large-scale microwave oven and carried out the rapid pyrolysis of larch-lumber (350 g) to isolate levoglucosan in about 3% yield from the lumber [19,20] (Scheme 2). This is an excellent method for the synthesis of levoglucosan on a semi-industrial scale. He has increased the weights of lumbers to 15 kg. It was found that other cellulose source such as used and filter papers also gave levoglucosan in 6 and 12% yields, respectively.

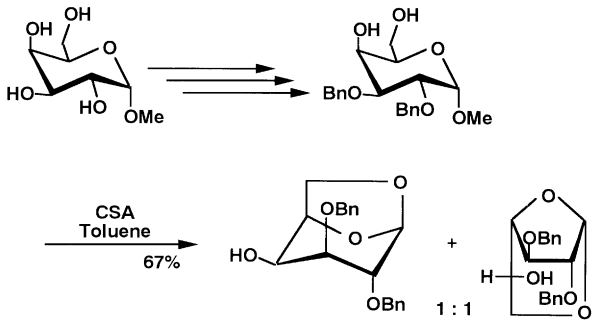
2.1.2. 1,4-Anhydro-α-D-glucopyranose

Benzylated 1,4-anhydro-α-D-glucopyranose was synthesized according to Micheel's method, namely, perbenzylation of cellulose followed by decomposition to a monomeric unit with conc. H_2SO_4 and subsequent intramolecular dehydration [21]. However, this method is a tedious procedure and the yield of the 1,4-anhydro-sugar was very low. A relatively simple method was reported in 1994 in which methyl α-D-glucopyranoside was converted to 1,4-anhydro-2,3-di-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose and 1,4-anhydro-3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranose though intermediates, 2,3-di-*O*-benzyl-6-*O*-pivaloyl-D-glucopyranose and 3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranose, respectively. These intermediates were treated with trimethylsilyl triflate at 0°C or *p*-toluenesulfonic acid in refluxing benzene to give the 1,4-anhydro-glucose derivatives in 57 or 54% yield [22]. For the investigations of ring-opening polymerizability on the hydroxyl-protective groups of 1,4-anhydro-glucose derivatives, we synthesized three new monomers, 1,4-anhydro-glucopyranose monomers, 1,4-anhydro-2,3,6-tri-*O*-methyl-α-D-glucopyranose, 1,4-anhydro-6-*O*-benzyl-2,3-di-*O*-methyl-α-D-glucopyranose, and 1,4-anhydro-2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranose, from methyl α-D-pyranoside in good yields by the intramolecular dehydration of 2,3,6-tri-*O*-substitued α-D-glucopyranosides with *p*-toluenesulfonic acid in benzene [23].

2.1.3. 1,6-Anhydro-2,3,5-tri-O-benzyl-α-D-galactofuranose

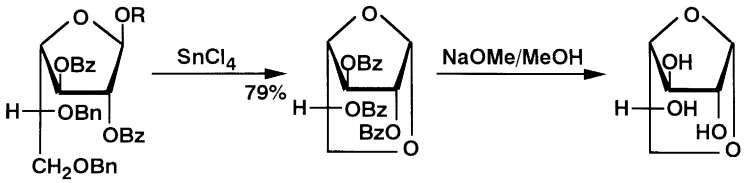
Previously, the hexofuranose-type monomer, 1,6-anhydro-2,3,5-tri-*O*-benzyl-α-D-galactofuranose, was obtained by benzylation followed by the column chromatography of a mixture of 1,6-anhydro-α-D-galactofuranose and 1,6-anhydro-α-D-galactopyranose, which were produced by the pyrolysis of D-galactose [24]. In a recent report, the intramolecular dehydration of methyl 2,3-di-*O*-benzyl-α-D-galactopyranose with an acidic catalyst, camphorsulfonic acid (CSA), in toluene gave equal amounts of 1,6-anhydro-galactopyranose and -galactofuranose [25], which were separated by column chromatography after benzylation to give 1,6-anhydro-2,3,5-tri-*O*-benzyl-α-D-galactofuranose (Scheme 3).

Synthesis of 1,6-anhydro-2,3,5-tri-*O*-benzoyl-α-D-galactofuranose has been achieved by stannic chloride catalyzed ring-closure of methyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactofuranoside in a good yield [26]. After debenzoylation followed by benzylation, 1,6-anhydro-2,3,5-tri-*O*-benzyl-α-D-galactofuranose was obtained. The starting methyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactofuranoside



Scheme 3.

was prepared from 1,2:3,4-di-*O*-isopropylidene-D-galactose by benzylation at the C6 position, followed by methanolysis and benzoylation of the product in 47% overall yield. Treatment of the starting methyl galactofuranoside derivative with stannic chloride in CH_2Cl_2 gave 1,6-anhydro-2,3,5-tri-*O*-benzoyl-α-D-galactofuranose in 79% yield, in which benzoyl groups were removed with sodium methoxide followed by acetylation to afford the corresponding acetate in a good yield (Scheme 4).



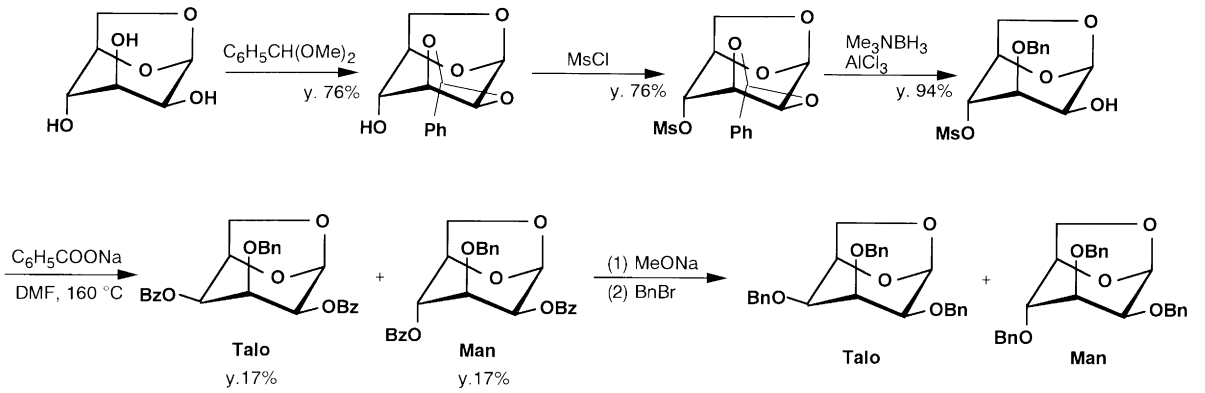
Scheme 4.

2.1.4. 1,6-Anhydro-2,3,4-tri-O-benzyl-β-D-talopyranose

As shown in Fig. 2, there are eight possible benzylated 1,6-anhydro-hexose monomers for the ring-opening polymerization. Among them, five 1,6-anhydro-hexose monomers were synthesized already and then polymerized to give polysaccharides with 1,6-stereoregularity. Recently, we synthesized a new monomer, 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-talopyranose, from 1,6-anhydro-β-D-mannopyranose [27]. Benzylidenation at the C2 and C3 hydroxyl groups and mesylation at the C4 hydroxyl group in 1,6-anhydro-β-D-mannopyranose followed by selective reduction of the benzylidene group gave 1,6-anhydro-2-*O*-benzyl-3-*O*-mesyl-β-D-mannopyranose in a good yield. The selective inversion at the C4 substitute was carried out with sodium benzoate in DMF to afford 1,6-anhydro-2,4-di-*O*-benzoyl-3-*O*-benzyl-β-D-talopyranose in 17% yield. After benzylation, the desired 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-talopyranose was obtained in a good yield (Scheme 5). The polymerization of the new anhydro-talopyranose monomer is now in progress.

2.2. Ring-opening polymerization of 1,6-anhydro-hexoses

The ring-opening polymerization of 1,6-anhydro-hexose monomers was first reported in 1966 by



Scheme 5.

Schuerch as described in Section 1 [3]. Since 1966, the polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose monomer (LGTBE) has been performed to give high molecular weight polymers. The polymerization proceeded with a trialkyloxycarbonium ion mechanism to give polymers with 1,6-α stereoregularity [9]. Other benzylated 1,6-anhydro-hexoses such as -mannose and -galactose gave the corresponding 1,6-α pyranosidic polysaccharides having high molecular weights. The 3,4-di-*O*-benzylated 1,6-anhydro-galactose monomer having a benzoyl group at the C2 position was polymerized

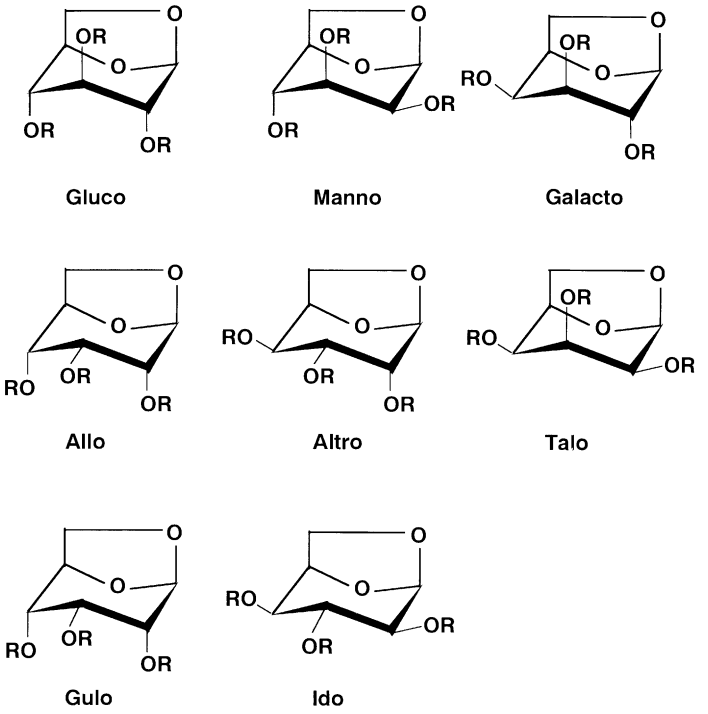


Fig. 2. 1,6-Anhydro-β-D-aldohexopyranose derivatives.

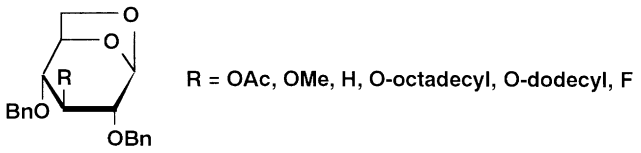


Fig. 3. Structure of 3-substituted 1,6-anhydro glucopyranose derivatives.

with the neighboring group effect of the C2 benzoyl group to give a 1,6-β galactopyranosidic polymer [28–30].

The biological functions of polysaccharides change significantly with a slight change in the chemical structure. In general, the chemical modifications of polysaccharides are difficult to proceed regioselectively because the three hydroxyl groups in a monosaccharide unit have similar reactivities. Therefore, regioselectively modified 1,6-anhydro monomers with two different hydroxyl-protective groups were polymerized by Kobayashi to afford stereoregular polysaccharides having a 3-*O*-substituent in the repeating unit. The substituents at the C3 position on 1,6-anhydro-2,4-di-*O*-benzyl-β-D-glucopyranose were acetyl [31], methyl [32], deoxy [33], octadecyl [34], dodecyl [34], and fluoride [35]. These modified polysaccharides were expected to model compounds for the investigations of specific biological recognitions (Fig. 3).

2.3. Ring-opening polymerization of 1,4-anhydro-pentoses

Benzylated 1,4-anhydro-pentose monomers, 1,4-anhydro-2,3-di-*O*-benzyl-α-D-ribopyranose (ADBR) [36,37], -xylopyranose (ADBX) [38], -lyxopyranose (ADBL) [39], and -L-arabinopyranose (ADBA) [40], were synthesized and polymerized with Lewis acid catalysts such as boron trifluoride etherate and phosphorus pentafluoride to give the corresponding 1,5-α-furanosidic polysaccharides after removal of the protective benzyl groups into hydroxyl groups (Fig. 4). The molecular weights were high. The polymerization to the 1,5-α furanosidic polymer might proceed through a trialkyl oxocarbonium ion intermediate as shown in Scheme 6 [36,37].

1,4-Anhydro-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-ribopyranose (ADSR) [41], -L-arabinopyranose (ADSA) [42], and -D-xylopyranose (ADSX) [43] were also polymerized with boron trifluoride etherate or phosphorus pentafluoride to give 1,5-α pentofuranosidic polymers, whose molecular weights were relatively smaller than those of the polymers from the benzylated monomers. It was found that the *ADSA* monomer gave a polymer having a complete 1,5-α furanosidic structure, although the *ADBA* monomer did not afford complete 1,5-α stereoregular polymers. The *tert*-butyldimethyl-silylated protective groups

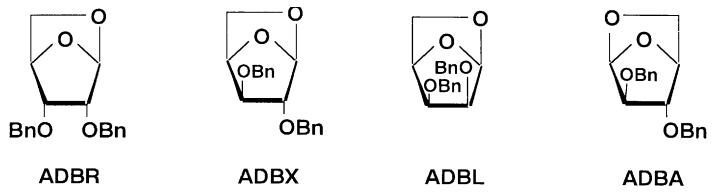
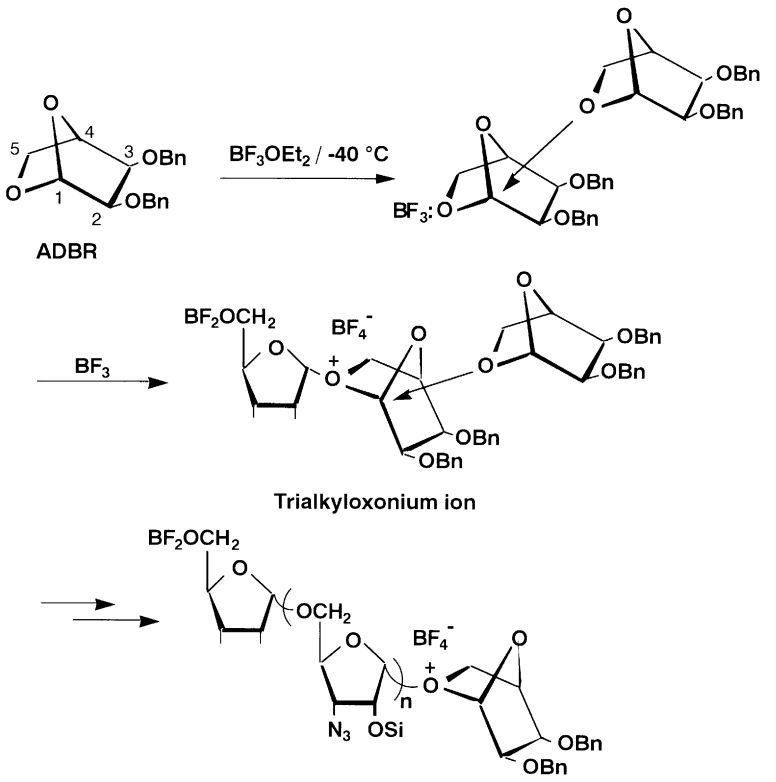


Fig. 4. Structure of benzylated 1,4-anhydro-pentose monomers. ADBR: 1,4-anhydro-2,3-di-*O*-benzyl-α-D-ribopyranose; ADBX: 1,4-anhydro-2,3-di-*O*-benzyl-α-D-xylopyranose; ADBL: 1,4-anhydro-2,3-di-*O*-benzyl-α-D-lyxopyranose; ADBA: 1,4-anhydro-2,3-di-*O*-benzyl-α-L-arabinopyranose.



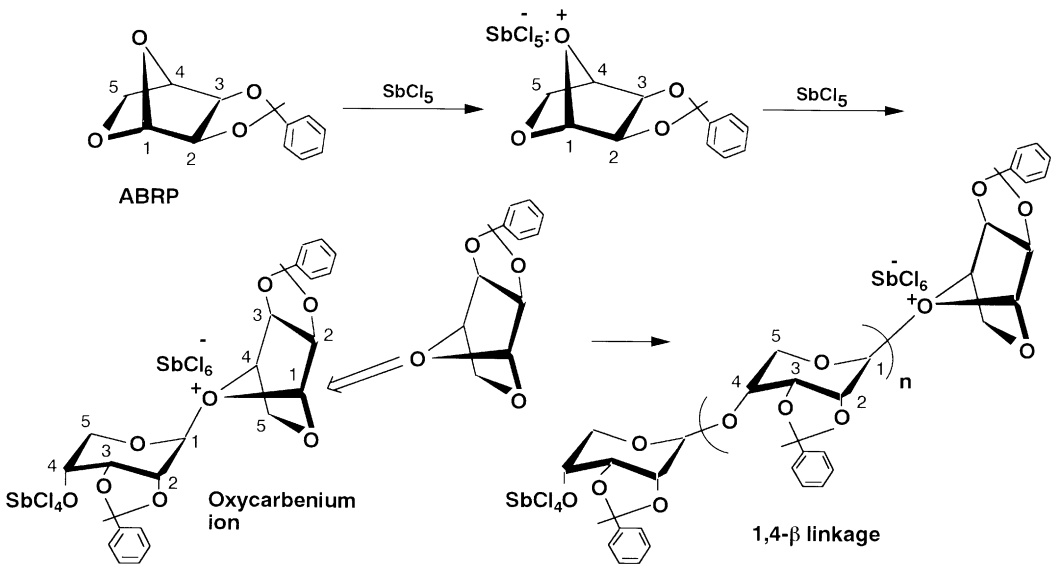
Scheme 6.

are removed easily to hydroxyl groups by tetra-*n*-butyl-ammonium fluoride in THF. We also attempted the ring-opening polymerization of 1,6-anhydro-2,3,4-tri-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranose monomer (LGTBS) with a Lewis acid catalyst. However, no polymer was obtained probably because of the steric hindrance of the silylated group and electron-donating effect at the C2 silylated protective group. Kobayashi described that a bulky axial substituent at the C2 position has a great effect on the polymerizability of 1,6-anhydro-sugars [44]. In addition, the 1,4-anhydro-furanose ring has a more highly strained system than that of the anhydro-pyranose ring system.

Uryu reported the synthesis of cellulose-type polysaccharides, 1,4-β-D-pyranosidic, where a new stereoregular 2,3-di-*O*-benzylidene-(1 → 4)-β-D-ribopyranan having a cellulose-type polymer backbone was synthesized by selective ring-opening polymerization of 1,4-anhydro-2,3-*O*-benzylidene-α-D-ribopyranose (ABRP) with antimony pentachloride as catalyst in methylene chloride [36,37]. 1,4-Anhydro-2,3-*O*-isopropylidene-α-D-ribopyranose (ADIR) was also polymerized with antimony pentachloride via the oxycarbenium ion mechanism to give stereoregular 2,3-*O*-isopropylidene-(1 → 4)-β-D-ribopyranan.

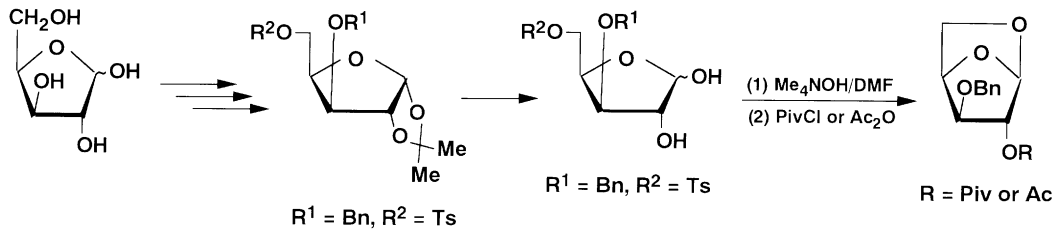
As mentioned above, the ADSR monomer gave only 1,5-α furanosidic polymers under any homopolymerization conditions. It was found that the selective ring-opening copolymerization of ABRP and ADSR monomers was accomplished by the $SbCl_5$ catalyst to give 1,4-β pyranosidic stereoregular polymers consisting of both benzylidenated and silylated ribopyranosidic units [45]. After selective desilylation, the benzylidene group remained intact in the resulting copolymer, which was subjected to

branching reactions to prepare branched polysaccharides. It has been considered that the copolymerization to 1,4-β-pyranosidic polymers with $SbCl_5$ catalyst proceeds by an oxonium ion mechanism as illustrated in Scheme 6. In the initiation step, $SbCl_5$ first coordinates to 1,4-linked oxygen (C1–*O*–C4) of ABRP monomer and then the 1,4-linked oxygen of the next approaching monomer attacks the propagating end from the backside direction of the $SbCl_5$-complexed ABRP to form an active initiating species with 1,4-β-pyranosidic structure (Scheme 7). Similarly to ABRP, ADSR can approach from its 1,4-linked oxygen to the active species to form a 1,4-β-linked copolymer backbone. In the copolymerization leading to 1,4-β-linked ribopyranose structure, the 1,4-β-linked initiating species can bind either an approaching ABRP or ADSR in such a way that the 1,4-linked oxygen of both monomers is cleaved to give both pyranose units. This tendency increased at a higher temperature.



Scheme 7.

For the synthesis of (1 → 5)-β-D-xylofuranan, two 1,4-anhydro-xylopynanose monomers, 1,4-anhydro-3-*O*-benzyl-2-*O*-pivaloyl- and 2-*O*-acetyl-1,4-anhydro-3-*O*-benzyl-α-D-xylopyranoses, were reported [46] (Scheme 8). Both monomers were obtained from D-xylose involving nine reaction steps



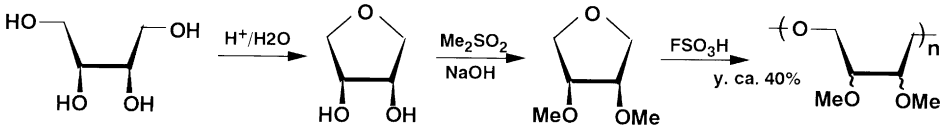
Scheme 8.

and in approximately 30–35% overall yields. The key reaction was the intramolecular nucleophilic attack of C1 oxoanion on the C5 position of 3-*O*-benzyl-5-*O*-(*p*-toluenesulfonyl)-α-D-xylofuranose to form a 1,5-acetal bond in high yield. This method should become a general procedure for 1,4-anhydro-α-D-xylopyranose derivatives having different substituents at the C2 and C3 positions. Previously, we reported a simple method of selective 2-*O*-protection of 1,4-anhydro-α-D-xylopynanose with a bulky *tert*-butyldimethylsilyl chloride to give 1,4-anhydro-3-*O*-azido-2-*O*-*tert*-butyldimethylsilyl-3-*O*-deoxy-α-D-ribopynanose after inversion at the C3 position in a good yield [47]. We found that this monomer gave a stereoregular 1,5-α furanosidic polymer by ring-opening polymerization with Lewis acid catalyst. The results of the polymerization will be described in detail in the later part of the synthesis of amino-polysaccharides.

2.4. Ring-opening polymerization (cyclopolymerization) of anhydrohexitol derivatives

2.4.1. cis-3,4-Dimethoxyoxolane and 1,4:2,5:3,6-trianhydro-D-mannitol

The ring-opening polymerization of highly strained small ring oxolans is of interest to synthesize polyethers. The ring-opening polymerization of 1,4-anhydro-2,3-di-*O*-methyl-D-erythritol (*cis*-3,4-dimethoxyoxolane) was carried out with fluorosulfonic acid as an initiator to give poly[oxy(2,3-dimethoxytetramethylene)] having number average molecular weights of around 1100 in ca. 40% yields [48] (Scheme 9).



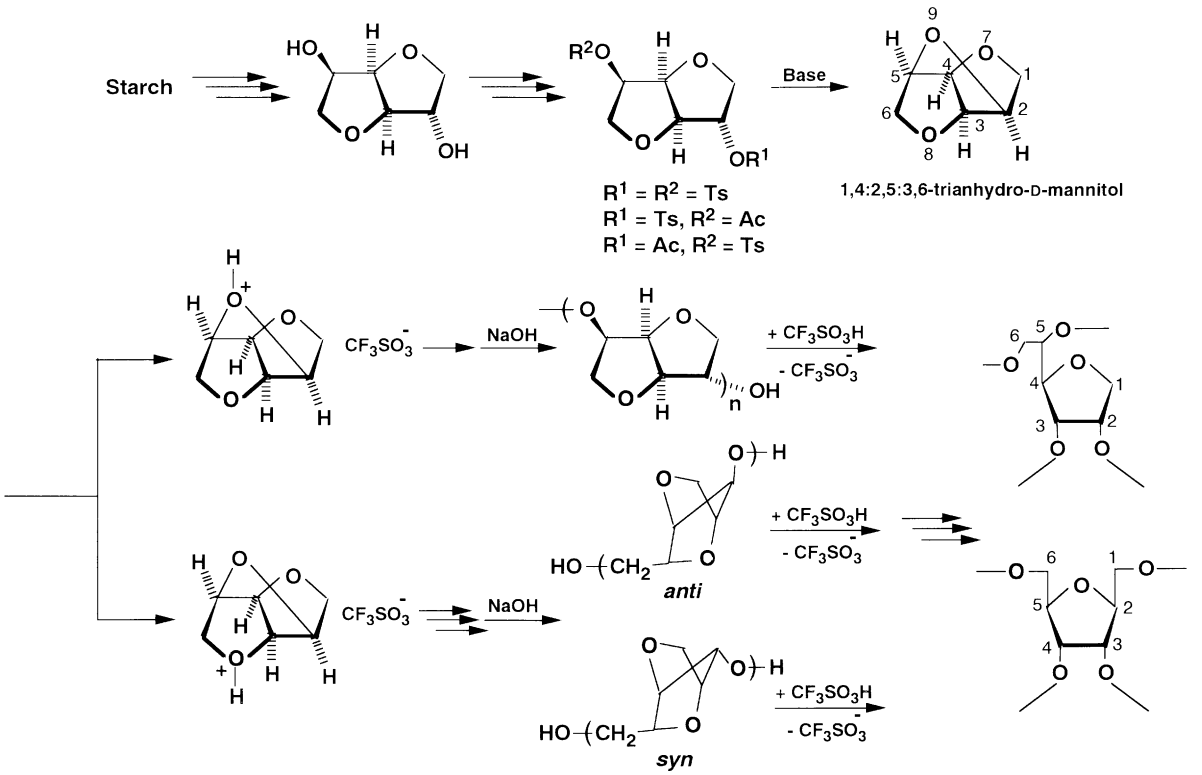
Scheme 9.

Also 1,4:2,5:3,6-trianhydro-D-mannitol was polymerized to yield polymeric carbohydrate oligomers ($\overline{DP}_n = 5–10$) with crosslinkings [49]. The starting monomer 1,4:2,5:3,6-trianhydro-D-mannitol was synthesized from starch (Scheme 10). Pertosylated 1,4:3,6-dianhydro-D-sorbitol was treated with sodium methoxide in anhydrous ethanol to give the trianhydro-mannitol in 56% yield. The polymerization was performed with 5–10 mol% of trifluoromethanesulfonic acid in CH_2Cl_2 at −30°C to produce crosslinked oligomers such as oligo(oxydianhydro-2,5-sorbitoldiyl) in the soluble and insoluble parts.

2.4.2. 1,2:5,6-Dianhydrohexitols

The cyclopolymerization of 1,2:5,6-dianhydrohexitol, which was reported by Kakuchi, is a similar polymerization method to the ring-opening polymerization of anhydro-sugar derivatives described in Sections 2.2 and 2.3. However, the difference between the cyclopolymerization and the ring-opening polymerization is not only the polymerization manner but also the resulting polymer structure. Although the monomeric units in the polysaccharides obtained by the ring-opening polymerization of anhydro-sugars were connected with the anomeric linkages, the cyclopolymerization of 1,2:5,6-dianhydrohexitols gave the polymer lacking anomeric linkages.

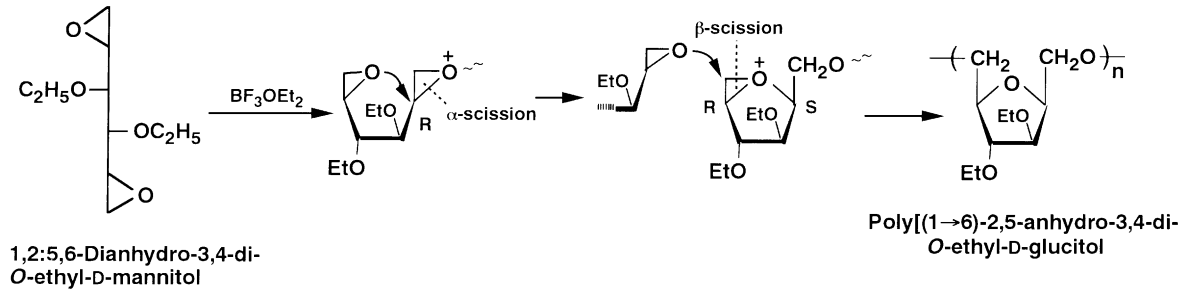
The cationic cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-*O*-ethyl-D-mannitol was carried out



Scheme 10.

with boron trifluoride etherate to give *n*-hexane-insoluble polymers, which was composed mainly of 2,5-anhydro-3,4-di-*O*-ethyl-D-glucitol as a cyclic constitutional unit [50,51]. In the *n*-hexane-soluble part, 1,6:2,5-anhydro-3,4-di-*O*-ethyl-D-glucitol was obtained as a large amount of product. 1,2:5,6-Dianhydro-3,4-*O*-isopropylidene-D-mannitol was polymerized with boron trifluoride etherate and tin tetrachloride to yield polymers with cyclic and acyclic units by the restricted free rotation of bonds between the carbons at the 3,4-positions.

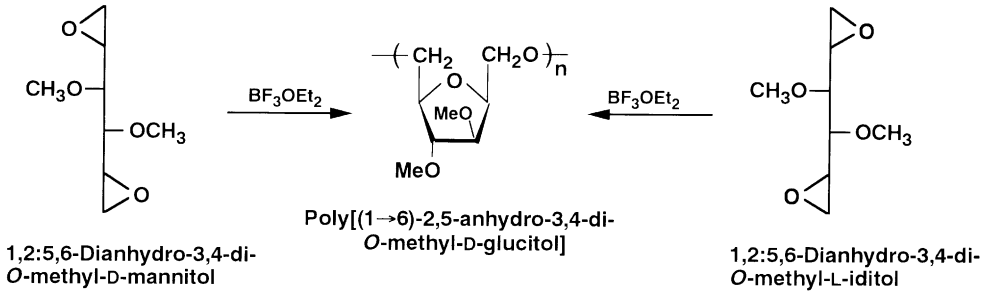
The cationic polymerization of 1,2:5,6-dianhydro-3,4-di-*O*-ethyl-D-mannitol should proceed through α,β-scission as shown in Scheme 11. The intermolecular cyclization occurs via the ring-opening of the



Scheme 11.

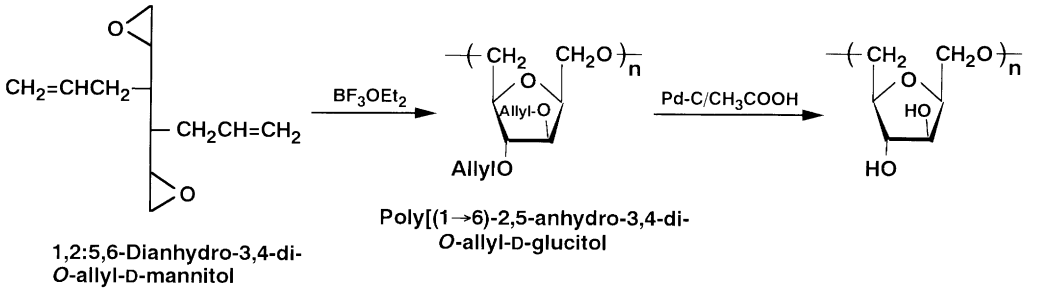
first epoxide with inversion (R → S) of the configuration by an S_N2 attack of the second epoxide function on the α-carbon of the former oxonium ion (α-scission). The ring-opening of the second epoxide takes place at the β-carbon with retention (R → R) of the configuration on the asymmetric carbon atoms, the carbon at which the attack is satirically favorable during the intermolecular propagation (β-scission) to give poly[(1 → 6)-2,5-anhydro-3,4-di-*O*-ethyl-D-glucitol].

Both 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-mannitol and -L-iditol were polymerized with boron trifluoride etherate in CH_2Cl_2 at −30°C to give the same polymer, poly[(1 → 6)-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol)] consisting of the five-membered constitutional unit in 68.3 and 45.4% yields, respectively (Scheme 12). The molecular weights were $\bar{M}_n = 1300$ and 1430, respectively [52]. By the polymerization for 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-mannitol in toluene at 0°C, the molecular weight increased to $\bar{M}_n = 3370$ but the yield decreased to 51.9%. When tin tetrachloride was used as a catalyst, both monomers gave the polymers below 10% yields and the molecular weights were 1430–2500.



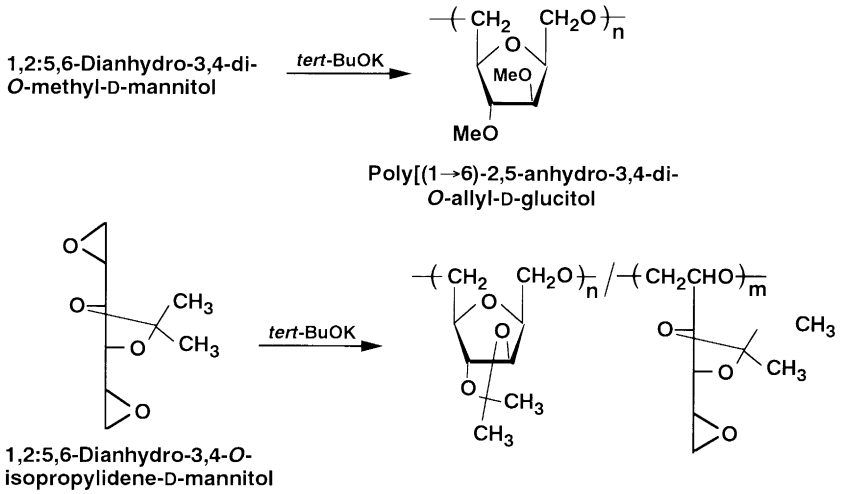
Scheme 12.

For the synthesis of deprotected polymer [53], poly[(1 → 6)-2,5-anhydro-D-glucitol)], the synthesis and cationic polymerization of 3,4-di-*O*-allyl-1,2:5,6-dianhydro-D-mannitol was carried out with boron trifluoride etherate in CH_2Cl_2 at −10°C to give poly[(1 → 6)-3,4-di-*O*-allyl-2,5-anhydro-D-glucitol)] having a molecular weight of $\bar{M}_n = 4890$ in 58.9% yield. The deallylation was performed with Pd–C catalyst in acetic acid/ethanol/water solvent system to form poly[(1 → 6)-2,5-anhydro-D-glucitol)] in 60.3% yield (Scheme 13).



Scheme 13.

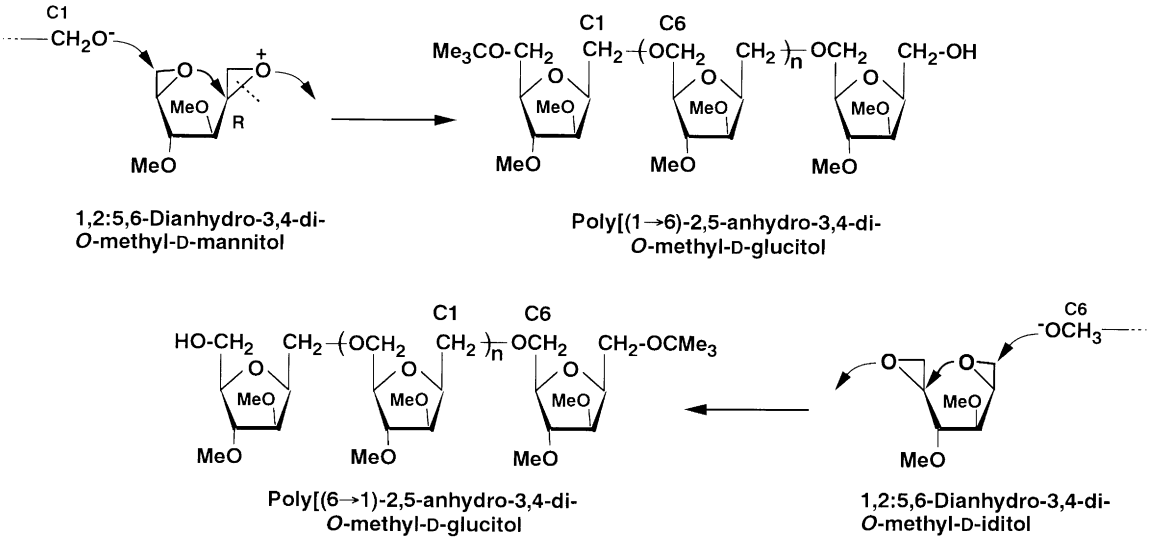
Instead of the cationic polymerization, the anionic cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-mannitol and 1,2:5,6-dianhydro-3,4-*O*-isopropylidene-D-mannitol was carried out with potassium *tert*-butoxide and potassium hydroxide as initiators [54] (Scheme 14). The polymerization of 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-mannitol proceeded through a regio- and stereoselective mechanism to afford a well-defined carbohydrate polymer consisting of 1,6-linked 2,5-anhydro-3,4-di-*O*-methyl-D-glucitol. When a [monomer]/[*tert*-BuOK] molar ratio of 40 was used in toluene at 60°C for 48 h, the polymer was obtained in 94.1% and the molecular weight was the highest, $\bar{M}_n = 12.9 \times 10^3$.



Scheme 14.

In addition, the effect of 18-crown-6 ether on the anionic polymerization of the mannitol monomer was examined [55]. The complexation of *tert*-BuOK with 18-crown-6 increased the initiator efficiency from 0.3 to 1.0, which was accompanied by enhancement of the apparent polymerization rate. In the two-step monomer addition, the polymerization continued to propagate on adding the second monomer. The degree of polymerization increased from 11.1 to 18.9, which corresponds to the calculated value of 20. These results suggest that the polymerization of 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-mannitol with *tert*-BuOK/18-crown-6 system proceeded in a "living" manner. On the other hand, 1,2:5,6-dianhydro-3,4-*O*-isopropylidene-D-mannitol gave a gel in the polymerization process. The restriction of free rotation at the C3 and C4 positions of the monomer strongly influences the degree to which its cyclization tends to proceed.

In the anionic cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-mannitol to the polymer consisting of 1,6-linked 2,5-anhydro-3,4-di-*O*-methyl-D-glucitol, the polymerization proceeded though the mechanism with β,α-scissions as shown in Scheme 15. For the intermolecular reaction, the growing alkoxy anion attacked the β-carbon of the first epoxide. For the intramolecular cyclization, the alkoxy anion produced from the first epoxide cleaved the α-bond of the second epoxide to form a polymer with a five-membered ring. The attack in the intramolecular reaction occurs not at the β-carbon but at the α-carbon.



Scheme 15.

The anionic cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-*O*-methyl-L-iditol was found to obtain (6 → 1)-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol as a five-membered repeating unit in good yields [56]. When the polymerization was carried out with *tert*-BuOH in toluene for 85 h, the yield and molecular weight of the resulting polymer were 98.6% and $\bar{M}_n = 3390$. Potassium hydroxide as a catalyst in THF gave the high molecular weight polymer of $\bar{M}_n = 6000$ but the yield decreased to 60.1%. As mentioned above, the cationic polymerization of this monomer gave no polymer with the five-membered repeating unit, but afforded a polymer with mixed structures of five-, six-, and seven-membered ring units, together with oligomers in fairly large quantities.

For the cationic polymerization of 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-glucitol with boron trifluoride etherate in CH_2Cl_2, the resulting polymer had the constitutional unit mainly of 2,5-anhydro-3,4-di-*O*-methyl-D-mannitol and the molecular weight of $\bar{M}_n = 3770$ [57]. For the anionic polymerization with *tert*-BuOK, the polymer having $\bar{M}_n = 5510$ consisted of two cyclic repeating units, 2,5-anhydro-3,4-di-*O*-methyl-D-mannitol and 2,5-anhydro-3,4-di-*O*-methyl-L-iditol. The anionic and cationic polymerizations of two meso dianhydro-hexitols, 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-allitol and -galactitol gave polymers with six-membered ring repeating units, distinct from 1,2:5,6-dianhydro-3,4-di-*O*-methyl-D-mannitol, -L-iditol, and -D-glucitol [58].

2.5. Ring-opening polymerization of glucopyranose 1,2,4-orthoester type monomers

For the synthesis of cellulose by the ring-opening polymerization of anhydro-glucose derivatives and by applying the neighboring group participation of a 2-*O*-acyl group of 2-*O*-benzoylated 1,6-anhydro-galactopyanose derivative to (1 → 6)-β-D-galactopyranan [28–30], 1,4-anhydro-2,3-di-*O*-benzyl-6-*O*-pivaloyl- and 1,4-anhydro-3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranoses were synthesized from methyl α-D-glucopyranoside [22] and then polymerized [59] (Fig. 5).

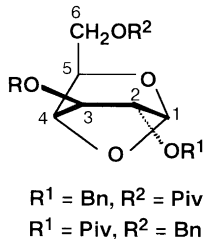
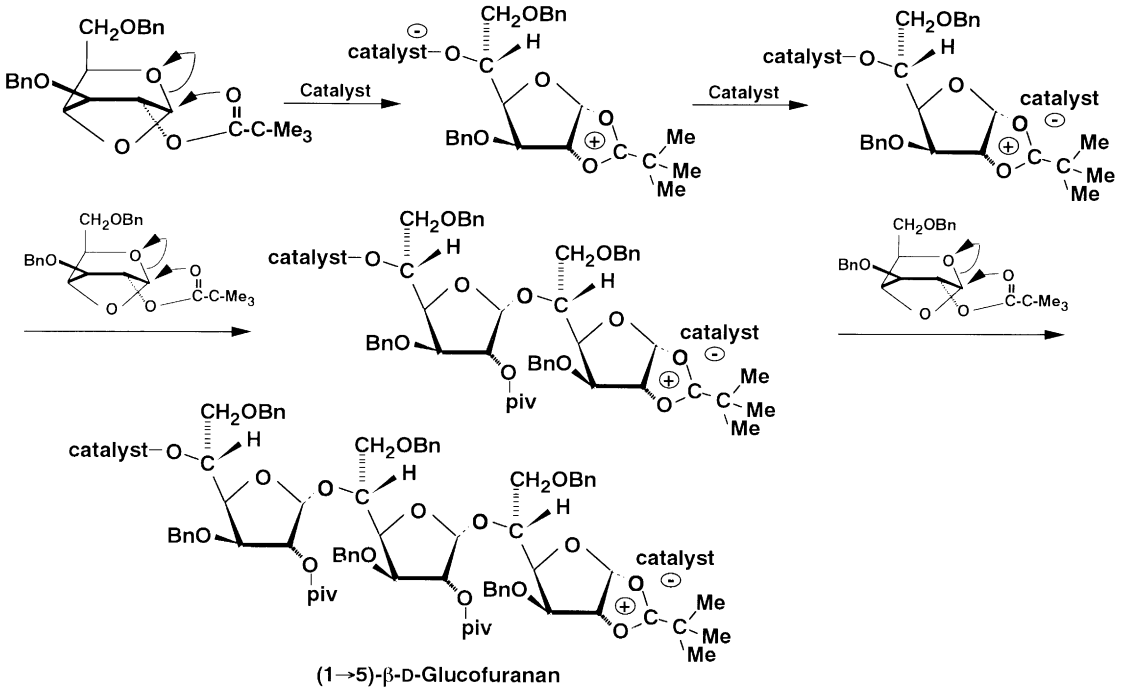


Fig. 5. Structure of 1,4-anhydro-2,3-di-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose and 1,4-anhydro-3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranose.

The ring-opening polymerization of 1,4-anhydro-2,3-di-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose was performed with phosphorus pentafluoride to give non-stereoregular polymers consisting of mainly (1 → 5)-α-D-glucofuranosidic units with an $[\alpha]_D^{25}$ of +84°. On the other hand, polymerization of 1,4-anhydro-3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranose with phosphorus pentafluoride produced a new stereoregular polysaccharide derivative, 3,6-di-*O*-benzyl-2-*O*-pivaloyl-(1 → 5)-β-D-glucopyranose, with an $[\alpha]_D^{25}$ of −66°. Debenzylation and depivaloylation afforded (1 → 5)-β-D-glucofuranan having free hydroxyl groups with an $[\alpha]_D^{25}$ of −204°. The mechanism yielding (1 → 5)-β-glucofuranan was described by the effect of neighboring group participation at the C2 acyl group as shown in Scheme 16. The catalyst coordinates with the oxygen of the 1,5-anhydro ring, the electron density of which increases due to the electron-donating benzyl group at the C6 position. This coordination would result in

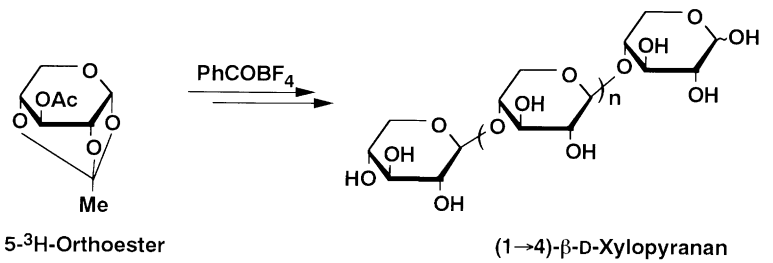


Scheme 16.

the formation of a (1 → 5)-β-D-furanose ring. The carbonyl oxygen of the pivaloyl group at the 2-*O*-position attacks C1 from the α-side to form a dioxacarbenium ion intermediate, and then the oxygen of the 1,5-anhydro ring of the next monomer attacks from the opposite side (β-side) of the intermediate to form (1 → 5)-β-D-furanose units.

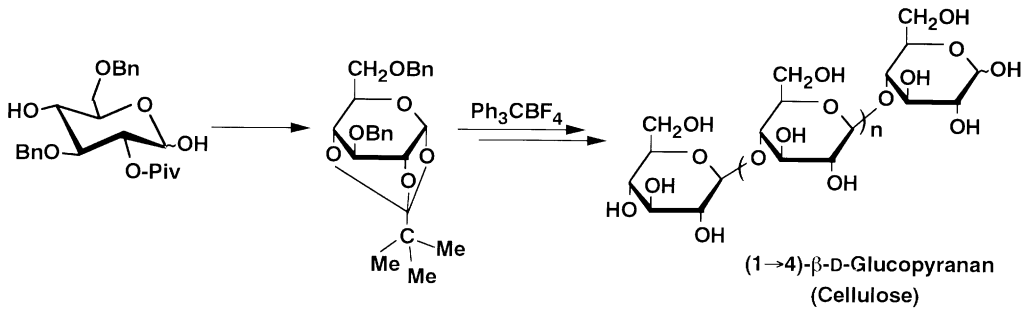
In order to investigate the effects of acyl group at the C2, C6, and C3 positions on the ring-opening polymerization, four monomers such as 1,4-anhydro-2,3-di-*O*-benzyl-6-*O*-pivaloyl-α-D-glucopyranose, 1,4-anhydro-3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-glucopyranose, 1,4-anhydro-3-*O*-benzyl-2,6-di-*O*-pivaloyl-α-D-glucopyranose, and 1,4-anhydro-6-*O*-benzyl-2,3-di-*O*-pivaloyl-α-D-glucopyranose were synthesized and polymerized under various reaction conditions [60]. It was concluded that both the pivaloyl group at the C2 and the benzyl group at the C3 positions are indispensable for yielding stereoregular (1 → 5)-β-D-glucofuranan derivatives with high molecular weights and that a substituent group at the C6 position hardly affects stereoregularity or polymerizability. These results indicate that the cationic ring-opening polymerization of bicyclic 1,4-anhydro-α-D-glucopyranose derivatives afforded preferentially (1 → 5)-D-glucofuranan rather than (1 → 4)-β-glucopyranan. It was impossible to synthesize stereoregular (1 → 4)-β-D-glucopyranan from the bicyclic 1,4-anhydro-α-D-glucopyranose derivatives.

One method for yielding highly regioselective 1,4-scission is utilization of orthoester derivatives having more reactive linkages than that of the substituted 1,4-anhydro-α-D-glucopyranose derivatives. Bochkov and Zaikov reported previously that the cationic polymerization was performed for the orthoester of D-xylose [61] (Scheme 17). The cationic polymerization of the orthoester was carried out with benzoylium tetrafluoride in CH_2Cl_2 at −35°C followed by deacetylation to give D-xylan (degree of polymerization, $\overline{DP}_n = 16$), 1,4-β-D-xylopyranan, in a 21% yield. From the results of the detailed structural analysis by means of partial and full hydrolysis, methylation analysis, and polarimetry, the polysaccharide contained about 14% of a 1 → 2-linkage and about 25% of all linkages had α-configuration.



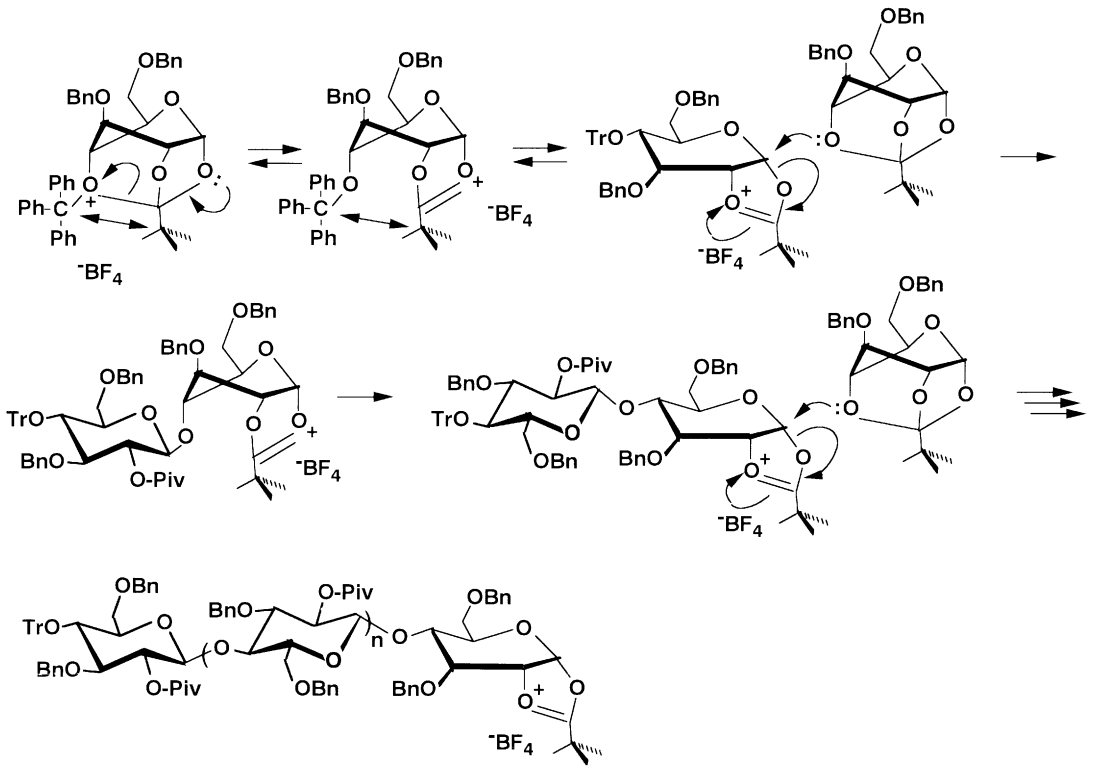
Scheme 17.

For the chemical synthesis of cellulose, Nakatsubo reported the synthesis and polymerization of an orthoester, 3,6-di-*O*-benzyl-α-D-glucose 1,2,4-orthopivalate to give (1 → 4)-β-D-glucopyranan, cellulose, after removal of the protective groups to hydroxyl groups [62] (Scheme 18). Polymerization of the orthoester by triphenyl carbenium tetrafluoroborate gave 3,6-di-*O*-benzyl-2-*O*-pivaloyl-β-D-glucopyranan with $[\alpha]_D^{25} = -37.2°$ and a number-average molecular weight of $\bar{M}_n = 8.3 \times 10^3$ ($\overline{DP}_n = 19.3$). Removal of the pivaloyl and benzyl groups and subsequent acetylation gave acetylated (1 → 4)-β-D-glucopyranan which was completely identical with cellulose triacetate prepared from low molecular weight cellulose. The synthesized acetylated (1 → 4)-β-D-glucopyranan was converted by deacetylation to cellulose, which has the cellulose-II crystal structure.



Scheme 18.

To investigate the substituent effects at the C3 and C6 positions on ring-opening polymerization of 3,6-di-*O*-benzyl-α-D-glucose 1,2,4-orthopivalate, four different orthoesters were prepared and polymerized under various reaction conditions, suggesting that the benzyl groups at the C3 position are indispensable for yielding stereoregular (1 → 4)-β-D-glucopyranan derivatives [63] (Fig. 6). The polymerization mechanism for yielding (1 → 4)-β-D-glucopyranan derivatives might be explained by the dioxalenium ion mechanism as illustrated in Scheme 19 rather than the trialkyloxonium ion mechanism. A dioxalenium ion was formed by the intramolecular backside attack of a lone pair orbital on the



Scheme 19.

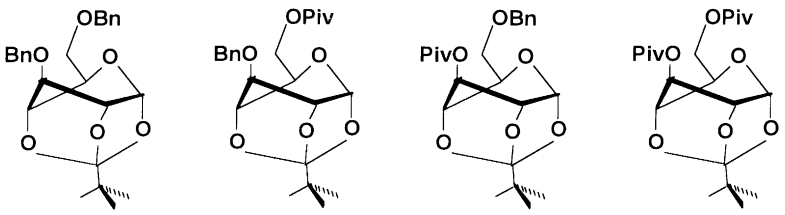


Fig. 6. α-D-Glucopyranose 1,2,4-orthopivalate derivatives.

C1- or C2-oxygens oriented anti-periplanar to the C4–*O*–C7 bond in preference to the attack of the monomer because of its instability caused by the large steric repulsion between the C4O-trityl and the C7-*tert*-butyl groups. The dioxalenium ion intermediate can then undergo an intermolecular reaction with the next monomer without any hindrance from the axial 3-*O*-benzyl group to produce a stable dimeric intermediate with the H-form. In the propagation step, the β-side attack of the next orthoester monomer on the reducing end of the elongating chain and conformational transformation of 3S1 into H-conformation occurred to afford a polymeric dioxalenium ion. Finally, one mole of water was introduced into the polymeric intermediate to give a completely stereoregular (1 → 4)-β-D-glucopyranan derivative.

In addition, to investigate the substituent effects of the orthoester group on the ring-opening polymerization, three orthoester derivatives, 3,6-di-*O*-benzyl-α-D-glucose 1,2,4-orthopropioate, 1,2,4-orthoacetate, and 1,2,4-orthobenzoate were synthesized and polymerized to give no complete 1,4-β-pyranosidic polymers. The orthoester monomers gave a mixture consisting of 1,4-β- and 1,2-β-pyranosidic units. However, the 1,4-β-stereoregularity was high, suggesting that the orthopivaloyl group of the starting monomer was indispensable for regiospecificity of the polymerization yielding only the 1,4-β-glycosidic bond, not the 1,2-bond [64]. The polymerization of orthopivalates having a 3-*O*-pivaloyl group yielded also no stereoregular polymers, a mixed polymer consisting of 1,4-β-, 1,2-α-, and 1,2-β-glucans because of the electron-withdrawing effects, but the 3-*O*-benzyl derivative gave stereoregular (1 → 4)-β-D-glucopyranan. Thus, it was concluded that both 3-*O*-benzyl and orthopivaloyl groups play important roles in the synthesis of stereoregular (1 → 4)-β-D-glucopyranan derivatives in the ring-opening polymerization of α-D-glucopyranose 1,2,4-orthoester derivatives.

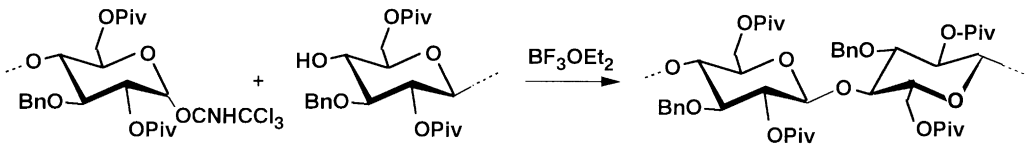
For the synthesis of (1 → 5)-β-D-galactofuranose, 3,6-di-*O*-benzyl-α-D-galactofuranaose 1,2,5-orthopivalate was synthesized from D-galactose by 10 reaction steps. Intramolecular orthoesterification was accomplished by treatment of 5-*O*-monochloroacetyl-2,3,6-tri-*O*-pivaloyl-D-galactofuranosyl chloride with thiourea in pyridine at 80°C to give the expected orthoester in a good yield with any side reaction [65]. The ring-opening polymerization of galactofuranose orthoester gave stereoregular (1 → 5)-β-D-galactofuranan.

2.6. Stepwise synthesis of cellulose

Cellulose is the most plentiful natural polymer on the earth in plants and is known to consist of D-glucose through the 1,4-β glycosidic linkage. Cellulose has drawn attention as a key polysaccharide because of renewable and biodegradable natural resources. The chemical synthesis of cellulose is very difficult in spite of its simple structure. In recent years, some reports have appeared for the chemical and enzymatic synthesis of cellulose.

Nakatsuo reported the synthesis of cello-octaose by a coupling reaction of cellotetraose derivatives. The key step of this cello-oligosaccharide synthesis is the β-glycosylation. Before the synthesis of cellulose, the β-glycosylation and stability of imidates of 3-*O*-benzyl-glucose derivatives protected by benzyl and acyl groups were examined [66]. The imidates protected by acyl groups up to the cellotetraoside were all stable during a purification by silica gel column chromatography [67]. The pivaloyl group at the C2 position of the glucose unit is more suitable than acetyl groups because β-glycosylation proceeded in higher yields and prevented orthoester formation. In addition, the imidate with the pivaloyl group only at the C6 position gave both α-and β-glucosides, whereas the imidate with the pivaloyl group at the C2 position gave only β-glucoside, suggesting that either 3,6-di-*O*-benzyl-2-*O*-pivaloyl and 3-*O*-benzyl-2,6-di-*O*-pivaloyl glucose derivatives are expected to be useful starting materials for the synthesis of cello-oligosaccharides.

The glycosylation between the cellotetraosyl donor and acceptor proceeded regioselectively in a one-step reaction under high vacuum to give a cello-octaose derivative [67] (Scheme 20). The coupling reaction was carried out by applying the neighboring group participation of a 2-*O*-pivaloyl group to form β-glycosidic linkage. After removal of pivaloyl, allyl, and benzyl protective groups with SeO_2–AcOH, NaOMe–MeOH, and $H_2/Pd(OH)_2$–C, respectively, peracetylated cello-octaose was obtained after acetylation. Finally, the cello-octaose acetate was deacetylated with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in 20% MeOH–CH_2Cl_2 to give pure cello-octaose.



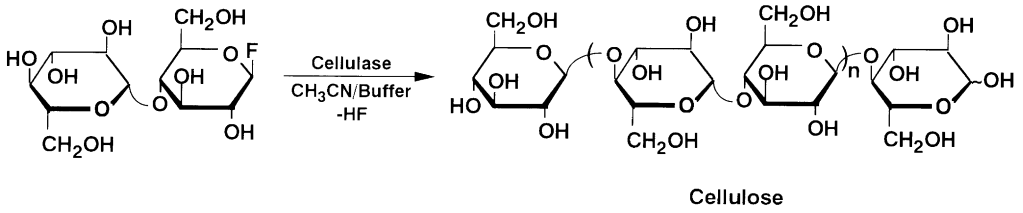
Scheme 20.

Furthermore, celloeicosamer (DP = 20) was obtained by a stepwise addition of cellotetraosyl α-trichloroacetoimidate derivative with cello-octaose derivative [68,69]. 1-*O*-Imidated cellotetraoside derivative was obtained easily and stably during purification procedures such as silica gel column chromatography. 1-*O*-Imidation of the cello-octaose derivative was very slow and the obtained imidated derivative was unstable. Thus, the 1-*O*-imidated cello-octaoside derivative was not used as the starting oligosaccharide moiety for the stepwise synthesis of cellulose.

2.7. Enzymatic polymerization to polysaccharides

Enzymatic polymerization of mono- or disaccharide derivatives is one of the important methods for the synthesis of not only polysaccharides with defined structures but also polyesters and polyamides [70–72].

In 1991, Kobayashi reported for the first time a completely novel approach for the synthesis of cellulose by a transglycosylation catalyzed by cellulase with β-cellobiosyl fluoride as a glycosyl donor [73]. The in vitro synthesis of cellulose was achieved by condensation of β-cellobiosyl fluoride as substrate for cellulase in a mixed solvent of acetonitrile-acetate buffer (pH 5, 5:1). The structure of the water-insoluble part of the products was confirmed by comparison with an authentic natural cellulose



Scheme 21.

sample with the use of solid ^{13}C NMR and IR spectroscopies as well as with a hydrolysis experiment. The synthetic cellulose was converted to the corresponding triacetate whose molecular weight was at least $\bar{M}_n = 6.3 \times 10^3$ ($\overline{DP}_n > 22$). (Scheme 21 — enzymatic polymerization of β-D-cellobiosyl fluoride with cellulase to cellulose). The binding site of the enzyme recognized the disaccharide donor, β-cellobiosyl fluoride, more readily than a monosaccharide, glucose, derivative. The disaccharide was the smallest substrate recognized by the enzyme. When cellobiose was used instead of cellobiosyl fluoride, only a small amount of oligomer was detected by HPLC, no polymerized product was obtained.

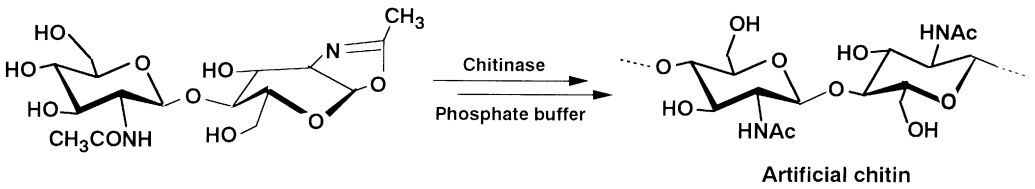
Malto-oligosaccharides were prepared by polycondensation of α-D-maltosyl fluoride using α-amylase as the catalyst in a mixed solvent of methanol-phosphate buffer (pH 7) [74]. In the presence of α-amylase from *Aspergillus oryzae*, α-D-maltosyl fluoride was condensed in a mixture of methanol and 0.05 M phosphate buffer (2:1) at room temperature for 1 h to give a mixture of maltooligosaccharides in quantitative yields. The polymerization proceeded in regio- and stereoselective manners to form a 1,4-α glycosidic linkage. Other substrates such as D-maltose, β-D-maltosyl fluoride, and α-D-glucosyl fluoride gave no condensation products.

Artificial xylan, (1 → 4)-β-D-xylopyranan, was also synthesized for the first time by the transglycosylation reaction catalyzed by cellulase using β-xylobiosyl fluoride as a substrate monomer [75]. The substrate monomer was polymerized smoothly to afford the corresponding polycondensation products. The structure was characterized by using CP/MAS solid ^{13}C NMR by comparison with the spectrum of a natural xylan and the treatment with xylanase, indicating clearly that the enzymatic polymerization of the fluoride monomer proceeded regio- and stereoselectively between xylobiose units to afford a stereoregular xylopyranan having a 1,4-β linkage. The number-average molecular weight was at least $\bar{M}_n 6.7 \times 10^3$ (DP > 23).

A convenient method for the synthesis of *N*,*N*′-diacetylchitobiose was developed by using a 1,2-oxazoline derivative of *N*-acetylglucosamine as new glycosyl donor for chitinase from *Bacillus* sp. [76]. The coupling reaction occurred by combining an oxazoline derivative as a glycosyl donor and *N*-acetylglucosamine as a glycosyl acceptor for chitinase to form peracetylated chitobiose having 1,4-β-linkage after acetylation in 43% yield.

This reaction was applied to the synthesis of chitin with higher molecular weights [77]. The ring-opening polyaddition of a chitobiose oxazoline derivative, a new monomer having a distorted structure with α-configuration at C1, was promoted exclusively by chitinase, giving rise to high molecular weight chitin, $\bar{M}_v = 4 \times 10^4$ in almost quantitative yields (Scheme 22). The glycosidic bond formation occurred in a regio- and stereoselective manner between chitobiose units with the inversion of configuration at C1 during the polymerization, giving rise to the stereoregular polysaccharide having 1,4-β linkage.

The synthesis of a polysaccharide from the substrate sucrose was reported by an enzyme-membrane reactor which was developed by commercially available capillary pore membranes with diameters of

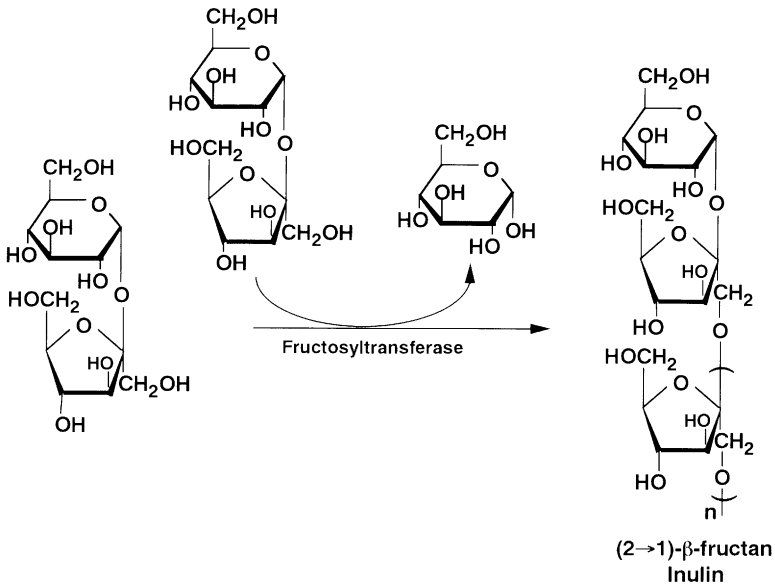


Scheme 22.

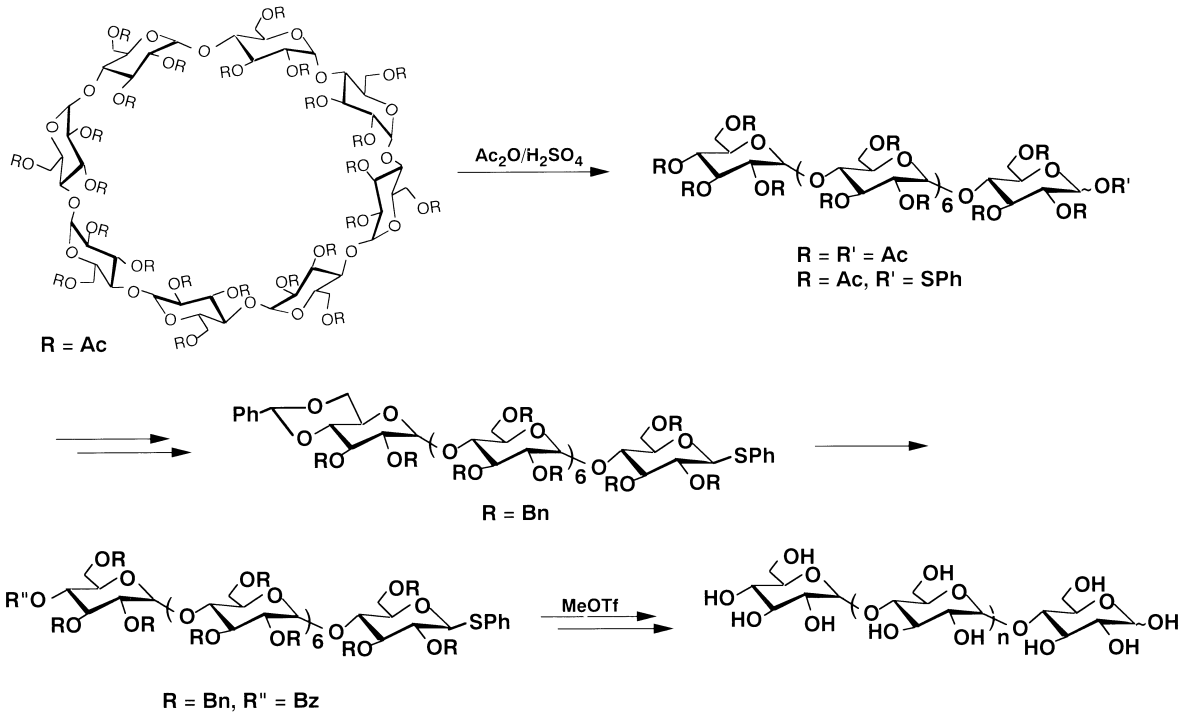
0.4, 1.0, and 3.0 mm as an immobilized carrier within the pores [78]. A grafted poly(2-amino ethyl methacrylate) layer with glutaraldehyde activation was suited particularly for coupling of fructosyltransferase from *Streptococcus mutans* to yield enzyme-membranes with high permeability and unchanged fructosyltransferase activities (Scheme 23). By using this membrane, sucrose was converted to (1 → 2)-β-D-fructan, inulin, having high molecular weights and narrow polydispersity of $\bar{M}_w/\bar{M}_n =$ 1.1. The fructosyltransferase catalyzed reaction involves cleavage of sucrose in glucose and fructose and subsequently coupling fructose via 1,2-β-linkage yielding inulin. After passing through the sucrose-containing phosphate buffer (200 g of sucrose per liter of buffer solution) for 2 h at 28°C, the ratio of inulin/glucose was 0.8 when the membrane with 3.0-μm pores was used. For a continuous enzymatic reaction driven by trans-membrane substrate flow, it was necessary to use membranes with rather spacious cylindrical pores (3.0 μm) to avoid their blocking by the produced inulin.

2.8. 1,4-α-Glucan from γ-cyclodextrin

Amylose was synthesized by the polycondensation and subsequent deprotection of a partially benzylated phenyl 1-thio-β-malto-octaoside having a hydroxyl group at the C4 position in the non-reducing



Scheme 23.



Scheme 24.

sugar unit [79], which octasaccharide was prepared by an acetolysis of peracetylated γ-cyclodextrin [80] (Scheme 24). The polycondensation proceeded with methyl triflate in diethylether through intermolecular glycosidation. The resulting polymer had 1,4-α glucopyranosidic structure similar to amylose and molecular weights of 10×10^3–18×10^3. After removal of benzyl groups to hydroxyl groups with sodium in liquid ammonia, stereoregular 1,4-α glucan was obtained and the structure was confirmed by NMR spectroscopy.

3. Synthesis of branched polysaccharides

In nature, there are many branched polysaccharides having specific biological activities such as antitumor activity as described later. However, it is difficult, in general, to know the structure–activity relationships, because these branched polysaccharides have a complex structure. Therefore, chemical synthesis of branched polysaccharides with defined structures is important to elucidate the relationship between biological activities and the polysaccharide structure. The ring-opening polymerization of anhydro-sugars is one of the useful methods for providing stereoregular polysaccharides. There are two methods for the synthesis of branched polysaccharides: (1) the ring-opening polymerization of anhydro-disaccharide or -oligosaccharide monomers; and (2) the glycosylation to synthetic and natural linear polysaccharides. Among these, the former is the better method for the synthesis of branched polysaccharides because precise regio- and stereoselective controls of the resulting branched structure,

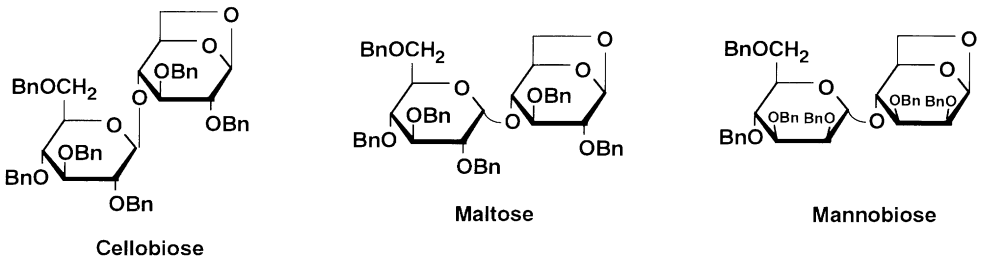


Fig. 7. 1,6-Anhydro-disaccharide monomers from cellobiose, maltose, and mannobiose.

and positions and proportions of branches are possible. However, the polymerizability of anhydro-disaccharide monomers reported previously is not so high without that of permethylated anhydro-lactose. Therefore, highly polymerizable anhydro-disaccharide monomers are required to lead to high molecular weight branched polysaccharides.

3.1. Ring-opening polymerization of anhydro-disaccharides and -oligosaccharides

There are four reports on the anhydro-disaccharide derivatives ring-opening polymerization, which gives comb-shaped polymers with a linear backbone and pendant monosaccharide units substituted on each sugar unit in the main chain.

3.1.1. 1,6-Anhydro-cellobiose

Benzylated 1,6-anhydro-cellobiose monomer, 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranose, was prepared from cellobiose octa-acetate by a conventional reaction [81]. The polymerization was carried out with phosphorus pentafluoride and benzoyl fluoride catalyst system (40 mol% each to monomer) in CH_2Cl_2 at $-60°C$ to give a stereoregular comb-shaped polysaccharide having a molecular weight of $\bar{M}_n = 7330$ consisting of a 1,6-α-linked glucopyranan main chain with each β-glucose unit at the C4 position in the repeating glucose unit in 72.1% yield. The yield of the polymer and its molecular weight were slightly increased by the use of benzoyl fluoride as cocatalyst. The large amounts of catalyst were necessary probably because of the many oxygens on the monomer which can coordinate with the catalyst. Maximum conversion (82%) was obtained at polymerization temperature at $-20°C$, but the molecular weight and specific rotation of the polymer decreased (Fig. 7).

3.1.2. 1,6-Anhydro-maltose

The polymerization of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-β-D-glucopyranose (benzylated 1,6-anhydro-maltose) was performed with the PF_5–PhCOF catalyst system (20–20 mol%) in CH_2Cl_2 for 100 h at $-60°C$ to afford a stereoregular comb-shaped polymer in 77% yield, which molecular weight and specific rotation were $\bar{M}_n = 13.4 \times 10^3$ and $[\alpha]_D^{25} = +96°$ (c2, $CHCl_3$), respectively [82]. After debenzylation, a comb-shaped polysaccharide having free hydroxyl groups with a high specific rotation, and $[\alpha]_D^{25} = +174°$ (c2, H_2O), which high value corresponds to a high 1,6-α-D stereoregularity in the main chain and 1,4-α-D-linkages on branches.

For the initiation studies, the copolymerization of *p*-methoxybenzylated 1,6-anhydro-glucose (M_1) with

benzylated 1,6-anhydro-maltose (M_2) was carried out under various polymerization conditions to give copolymers with a linear backbone and randomly distributed single glucose units as side chains. The yields of copolymers decreased in comparison with those of homopolymers [83]. The monomer reactivity ratio was calculated to be $r_1 = 1.91 \pm 0.35$, $r_2 = 0.28 \pm 0.25$, and $r_1 = 2.21 \pm 0.15$, $r_2 = 0.21 \pm 0.10$, for polymerizations catalyzed by 10 and 20 mol% of PF_5, respectively, suggesting that the copolymers obtained should be random copolymers.

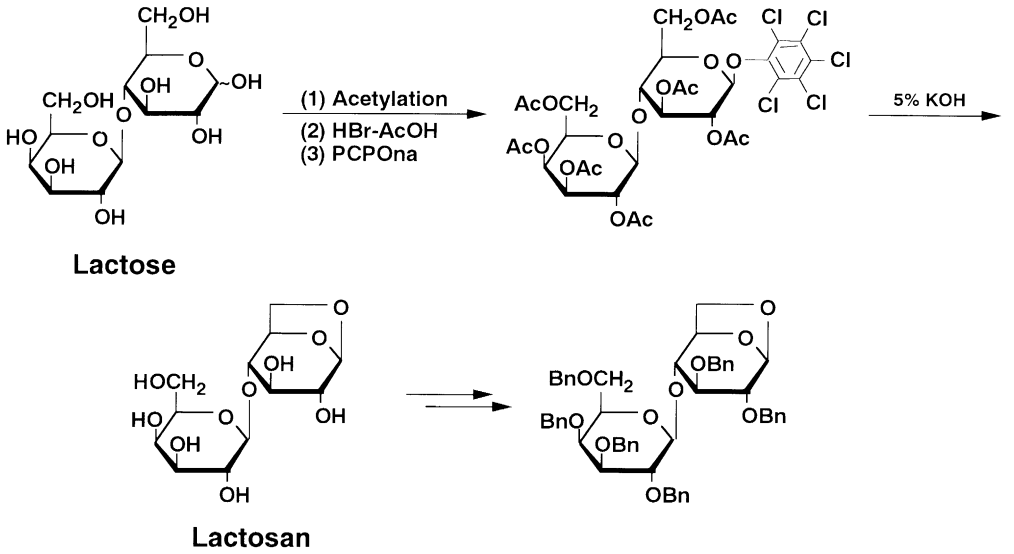
3.1.3. 1,6-Anhydro-mannobiose

The disaccharide monomer, 1,6-anhydro-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-2,3-*O*-isopropylidene-β-D-mannopyranose was obtained via glycosidation of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose with 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-mannopyranose using *p*-toluenesulfonic acid as catalyst and subsequent transformation of the isopropylidene group to benzyl group. The ring-opening polymerization of benzylated 1,6-anhydro-mannobiose monomer was carried out using 20 mol% PF_5 initiator in CH_2Cl_2 for 26 h at −60°C to give a 1,6-α-linked polymer in 70% yield. The molecular weight was relatively high, $\bar{M}_n = 23 \times 10^3$ and $\bar{M}_w/\bar{M}_n = 2.2$. After deprotection with sodium in liq. ammonia, 4-*O*-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranan with $\overline{DP}_n = 28$ was obtained [84].

3.1.4. 1,6-Anhydro-lactose

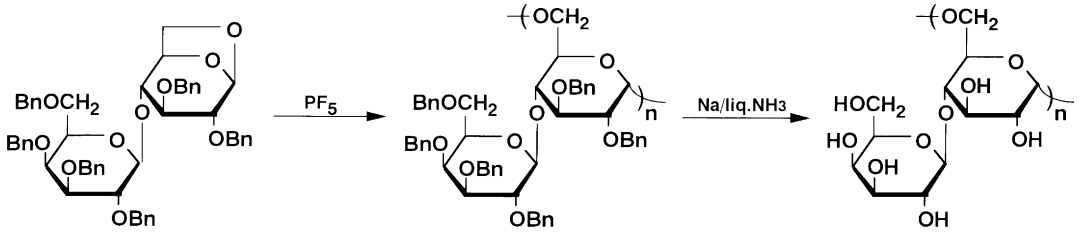
(+)-Lactose [4-*O*-(β-D-galactopyranosyl)-D-glucose] is one of three natural disaccharides, lactose, sucrose, and trehalose, and contained in milk at about 5 wt%. Therefore, lactose is a readily available natural polysaccharide as a by-product of cheese manufacturing. To elucidate the steric hindrance of the hydroxyl-protective groups on the ring-opening polymerizability, new anhydro-disaccharide monomers, methylated and *tert*-butyldimethylsilylated 1,6-anhydro-β-D-lactose was synthesized and polymerized [85]. It was found that the methylated lactose monomer gave the highest molecular weight of polymers among the 1,6-anhydro-disaccharide monomers. The methylated monomer was polymerized with 40 mol% of PF_5 in CH_2Cl_2 at −78°C to afford a stereoregular polymer having $\bar{M}_n = 355 \times 10^3$, which had a 1,6,-α-linked glucopyranose main chain with a β-D-galactopyranose at the C4 position of the main chain glucose unit. When the polymerization was carried out with 20 mol% of PF_5 at −40°C, the yield of polymer was 76.2% but the molecular weight distribution was not narrow, $\bar{M}_w/\bar{M}_n = 26.1$, suggesting that depolymerization might occur by both an excess amount of catalyst and a relatively high temperature. The polymerization with 15 mol% of $SbCl_5$ was revealed to afford a polymer with a high molecular weight of $\bar{M}_n = 69 \times 10^3$ and the narrow molecular weight distribution of $\bar{M}_w/\bar{M}_n = 1.14$. Although the ring-opening polymerization of *tert*-butyldimethylsilylated 1,6-anhydro-β-D-lactose was performed under the same polymerization conditions as those used with the methylated monomer, no polymer was obtained and the monomer was recovered. These results indicate that the polymerizability of the 1,6-anhydro-lactose monomer was affected to a large extent by the steric hindrance and the electric effect of the hydroxyl-protective groups (Scheme 25 — synthesis of 1,6-anhydro-lactose monomer, 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranose).

In addition, the benzylated 1,6-anhydro-β-D-lactose monomer was synthesized and the ring-opening polymerization was attempted to synthesize comb-shaped polysaccharides having free hydroxyl groups [86]. As described later, it was found that sulfonated lactopyranans had specific anti-HIV and blood anticoagulant activities. The benzylated monomer was highly purified by HPLC. Pentafluoro phosphate



Scheme 25.

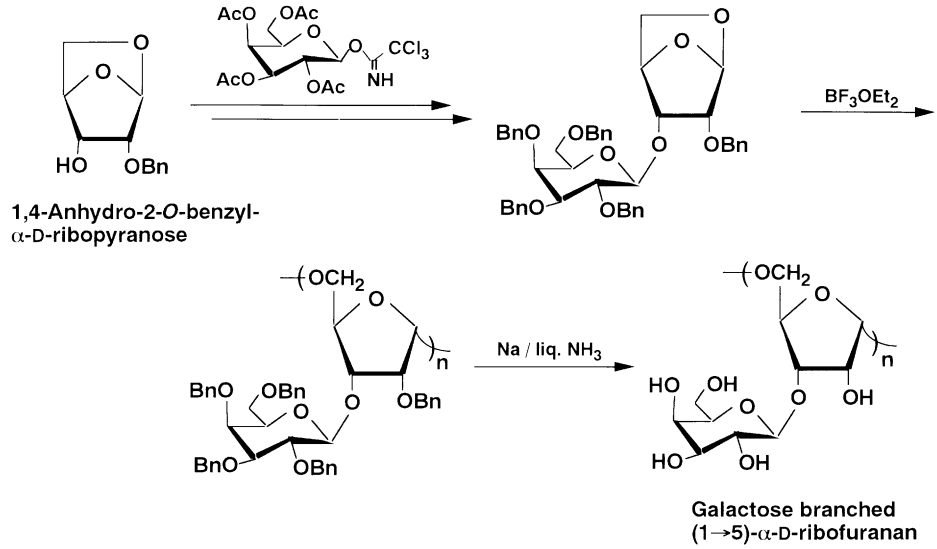
(20 mol%) in CH_2Cl_2 at −60°C gave a polymer having $\bar{M}_n = 5.5 \times 10^3$ in 68.5% yield. At the polymerization temperature of −78°C, the molecular weight increased to $\bar{M}_n = 7.3 \times 10^3$ but the yield decreased to 32.9%. Other Lewis acid catalysts such as $SbCl_5$, $BF_3{\cdot}OEt_2$, $SnCl_4$ worked ineffectively. After debenzylation with sodium in liq. ammonia, 4-*O*-β-D-galactopyranosyl-(1 → 6)-α-D-glucopyranan was obtained. The copolymerization of benzylated 1,6-anhydro-β-D-lactose with benzylated 1,6-anhydro-β-D-glucose afforded copolysaccharides having various proportions of branches on the main chain after debenzylation (Scheme 26).



Scheme 26.

3.1.5. 1,4-Anhydro-ribodisaccharide [87]

By using the high ring-opening polymerizability of 1,4-anhydro-α-D-ribopyranose derivative, a new ribo-disaccharide monomer, 1,4-anhydro-2-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-ribopyranose was synthesized by the glycosylation at the C3 position of 1,4-anhydro-2-*O*-benzyl-α-D-ribopyranose with 2,3,4,6-tetra-*O*-acetyl-1-*O*-trichloroacetimidoyl-α-D-galactopyranose. After removal of acetyl groups and subsequent benzylation, the ribo-disaccharide monomer was obtained and then highly purified by HPCL. It was revealed that the polymerization with 10 mol% of



Scheme 27.

$BF_3{\cdot}OEt_2$ at −40°C proceeded rapidly within 15 min to give a methanol-insoluble polymer of high molecular weight, $\bar{M}_n = 43 \times 10^3$ in 73.0% yield. The polymer had a 1,5-α stereoregular polymer with a β-D-galactose unit in the repeating unit. The phosphorus pentafluoride catalyst gave the polymer in high yield of 87% but the molecular weight was not so high, $\bar{M}_n = 11 \times 10^3$. The high polymerizability of the anhydro ribo-disaccharide monomer was probably due to the large strained 1,4-anhydro-ribopyranose (= 1,5-anhydro-ribofuranose) ring structure on the main chain. After debenzylation, stereoregular 3-*O*-β-D galactopyranosyl-(1 → 5)-α-D-ribofuranan was obtained (Scheme 27; Fig. 8).

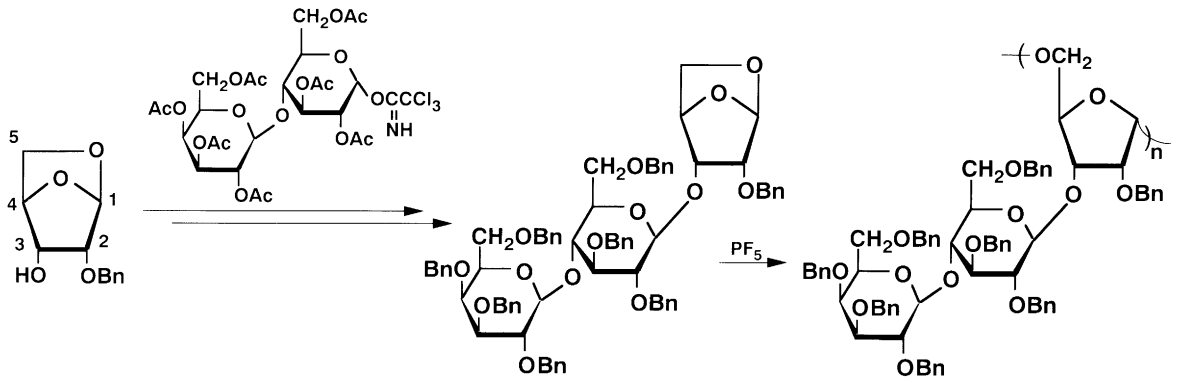
3.1.6. 1,4-Anhydro-ribotrisaccharide [88]

Quite recently, we prepared a new 1,4-anhydro-ribotrisaccharide monomer, 1,4-anhydro-2-*O*-benzyl-3-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-D-ribopyranose, by the glycosylation of 1,4-anhydro-2-*O*-benzyl-α-D-ribopyranose with 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactopyranosyl trichloroacetimidate. It was found for the first time that the anhydro trisaccharide monomer was polymerized under ordinary polymerization conditions to give a branched polysaccharide with (1 → 4)-α-D-ribofuranan main chain having β-D-lactose branches at the C3 position on the repeating ribofuranose unit. Polymerization with 10 mol% of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at −40°C gave 2-*O*-benzyl-3-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-β-D-lactopyranosyl)-(1 → 5)-α-D-ribofuranan having $\bar{M}_n = 6.5 \times 10^3$ in 62% yield (Scheme 28).

3.1.7. 1,6-Anhydro-deoxydisaccharide

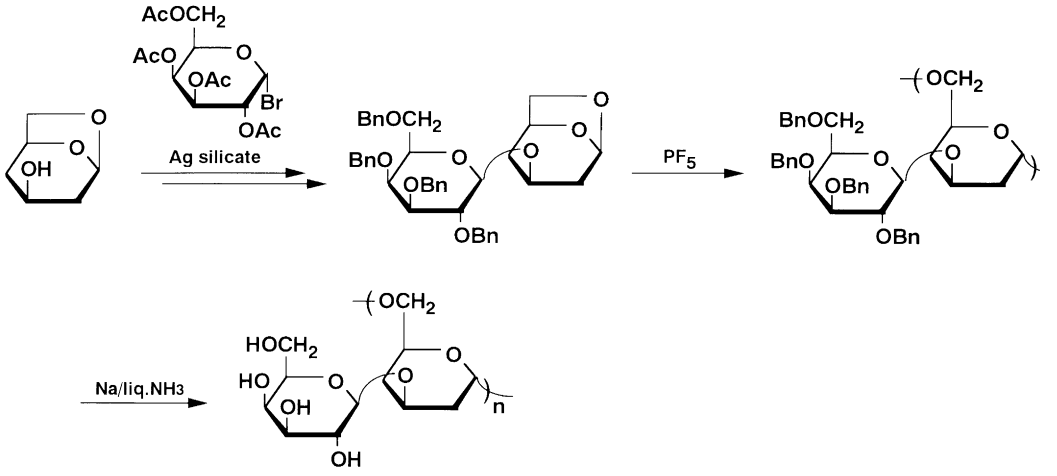
An anhydro disaccharide monomer increases the ring-opening polymerizability with a decrease of the steric hindrance because of the substituents on the structure of the anhydro deoxysugar moiety. Further, the trialkyloxonium ion of the growing terminal of the anhydro-deoxy moiety should be less sterically hindered, more basic, and more nucleophilic than that in the corresponding benzylated anhydro monomers.

For high molecular weight comb-shaped polysaccharides, the anhydro-deoxydisaccharide monomer, 1,6-anhydro-2,4-dideoxy-3-*O*-(β-D-galactopyranosyl)-α-D-*threo*-hexopyranose, was prepared by the



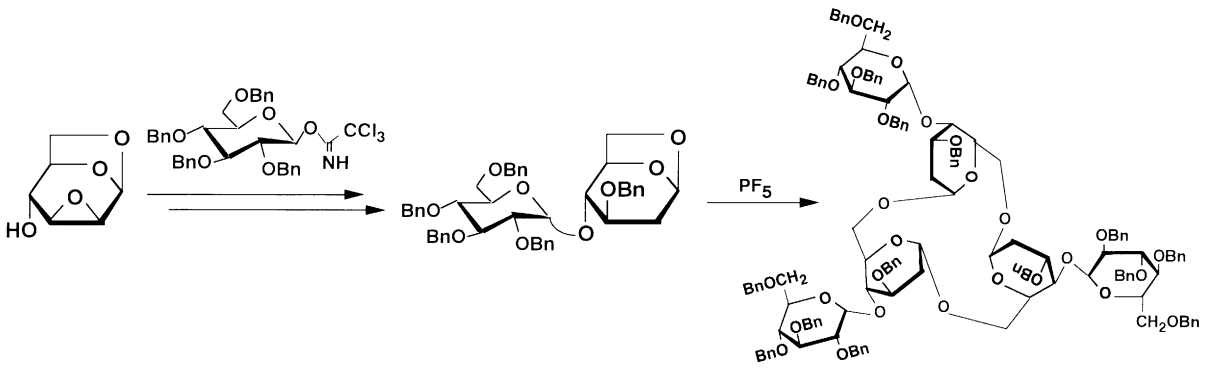
Scheme 28.

glycosylation of 1,6-anhydro-2,4-dideoxy-β-D-*threo*-hexopyranose with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide [89]. The polymerization was carried out with 20 mol% of PF_5 at −78°C to give a polymer with $\bar{M}_n = 27 \times 10^3$ in 67% yield. Anhydro disaccharide derivatives seem less reactive in polymerization than the corresponding anhydro monosaccharide derivatives. The polymer was debenzylated to give 2,4-dideoxy-3-*O*-(β-D-galactopyranosyl)-(1 → 6)-α-D-*threo*-hexopyranan (Scheme 29).



Scheme 29.

Another 1,6-anhydro-dexoydisaccharide monomer, 1,6-anhydro-3-*O*-benzyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-β-D-*arabino*-hexopyranose, was prepared by a glycosyl acceptor, 1,6:2,3-dianhydro-β-D-mannopyranose, with a glycosyl donor, 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate. The ring-opening polymerization was carried out with 20 mol% of PF_5 in CH_2Cl_2 at −60°C to give a mixture of a comb-shaped polymer and a branched trimer in the proportion of 60:40 mol%. The molecular weight of the linear product was 10.9×10^3. When the polymerization was



Scheme 30.

performed for 48 h at the higher temperature of 0°C, the trimer was only obtained in 89% yield [90] (Scheme 30).

3.2. Glycosylation to synthetic linear polysaccharides

Some reports have appeared on the glycosylation of synthetic linear polysaccharides such as dextran and ribofuranan. The branched polysaccharides were used to investigate lectin–carbohydrate reactions and model polysaccharides in the fields of allergy, enzymology, and immunology. In addition, the

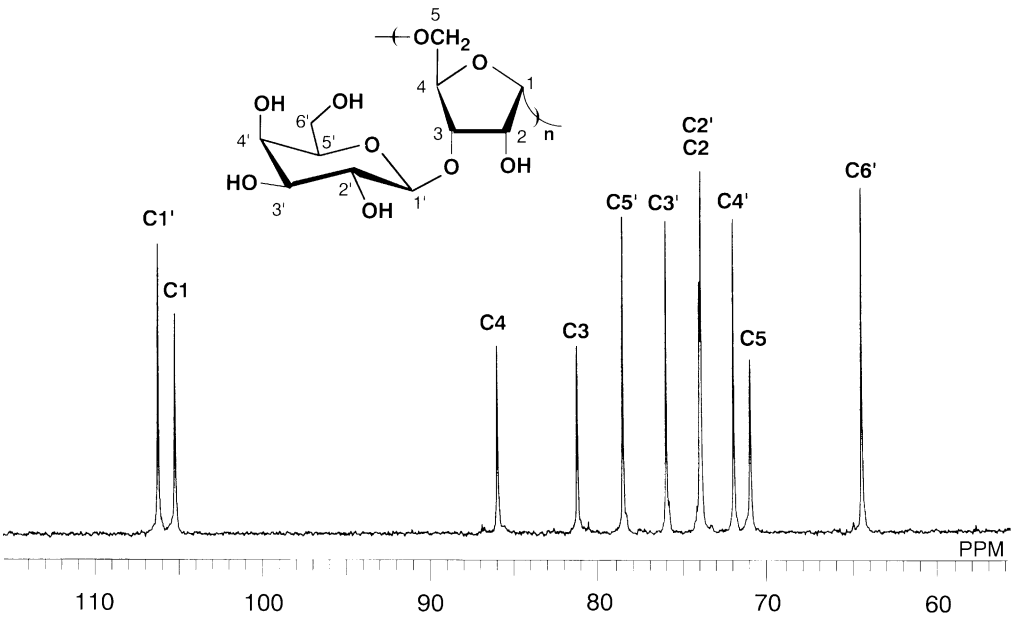


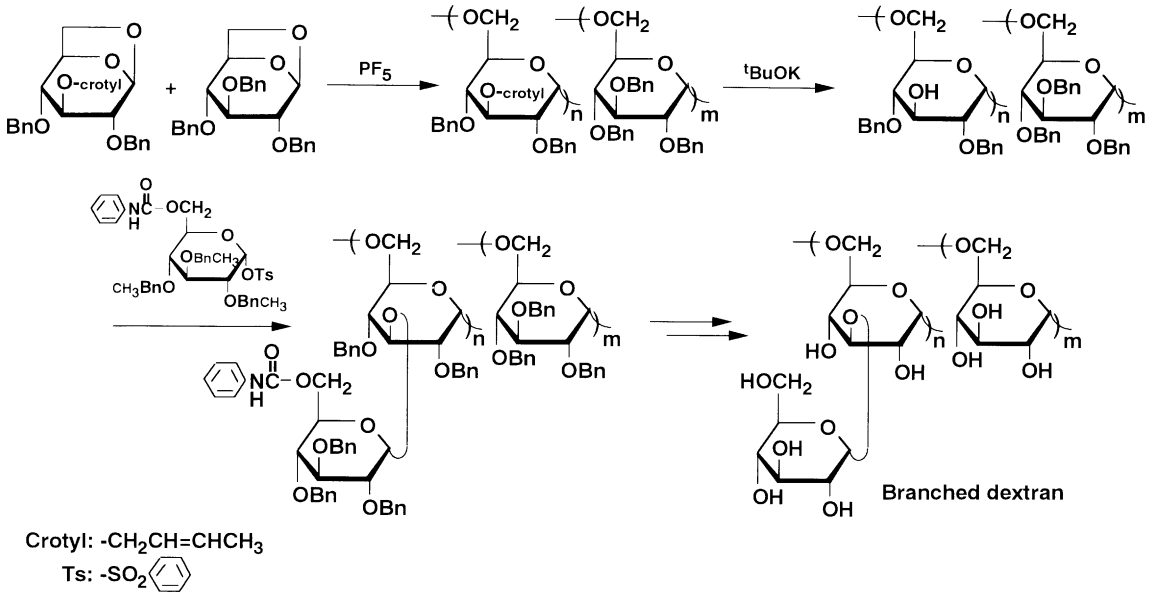
Fig. 8. ^{13}C NMR spectrum of 3-*O*-β-D-galactopyranosyl-(1 → 5)-α-D-ribofuranan having $M_n = 12.7 \times 10^3$ and $[\alpha]_D = +91.6°$ (c1, H_2O) (in D_2O, 37°C).

branched polysaccharides after sulfonation are expected to be specific biological activities such as anti-HIV and blood anti-coagulant activities as described later.

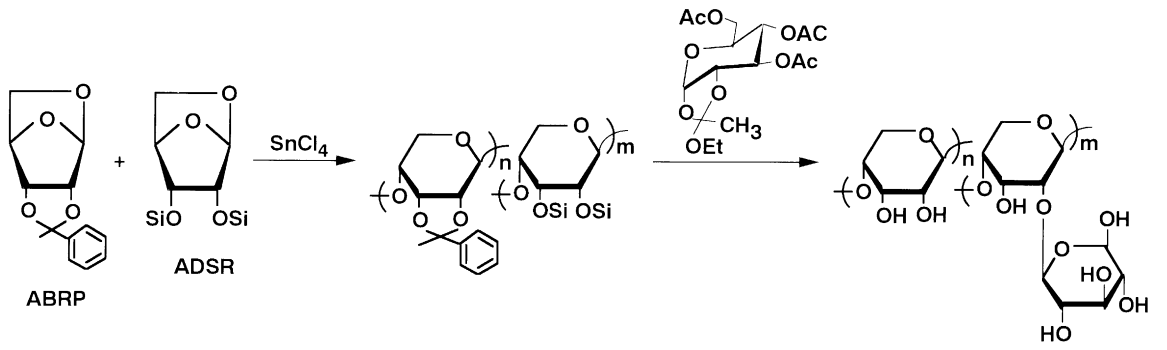
3.2.1. -O-branched dextran

In 1979, Ito and Schuerch reported for the first time the synthesis of artificial branched polysaccharide [91]. The ring-opening polymerization of 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-but-2-enyl-β-D-glucopyranose was carried out with PF_5 catalyst in CH_2Cl_2 at −60°C to give a 1,6-α-linked stereoregular polymer with a high molecular weight of $[\eta] = 1.25$ dl/g. Synthetic branched dextran with 3-*O*-(α-D-glucopyranosyl) side chains was prepared by the selective decrotylation with potassium *tert*-butoxide in benzene in the presence of 18-crown-6 at 90°C followed by α-D-glycosylation of 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-α-D-glucopyranose and then removal of protective groups to hydroxyl groups. The glycosylation proceeded completely by using a large excess of the tosyl derivative (10 equiv.). It was 0.97 for the mole fraction of the branched glucose unit in the main chain. The copolymerization of the 3-*O*-crotyl monomer with 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose followed by the branching reaction gave branched dextrins with different degrees of branching (Scheme 31).

Instead of the 3-*O*-crotyl group, Uryu used a *tert*-butyldimethylsilyl group as the 3-*O*-protective group on 1,6-anhydro-β-D-glucopyranose [92]. The ring-opening polymerization of 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranose gave 2,4-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-(1 → 6)-α-D-glucopyranan having $\bar{M}_n = 56 \times 10^3$ in 87.5% yield. Desilylation with tetrabutylammonium fluoride in THF followed by glycosylation with 3,4,6-tri-*O*-acetyl-β-D-mannose-1,2-(methyl orthoacetate) afforded 3-*O*-α-mannosylated dextran after deprotection. When the glycosylation was carried out with 3,4,6-tri-*O*-acetyl-α-D-glucopyranose-1,2-(*tert*-butyl orthoacetate), 3-*O*-β-glucosylated dextran was obtained [93].



Scheme 31.

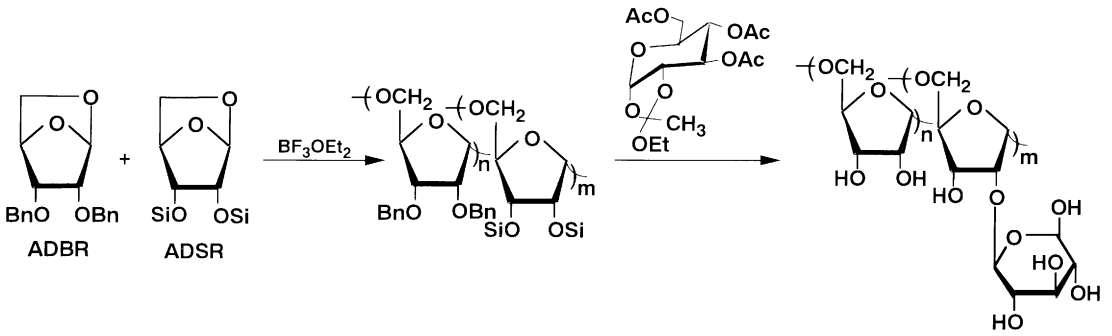


Scheme 32.

3.2.2. Branched ribofuranan and ribopyranan

Stereoregular cellulose-type copolymers, (1 → 4)-β-D-ribopyranan derivatives, were prepared by the selective ring-opening copolymerization of 1,4-anhydro-benzylidene- and -2,3-de-*O-tert*-butyldimethylsilyl-α-D-ribopyranoses (13.2 and 20.7 mol%) with $SbCl_5$ as a catalyst at 0°C [94]. By homopolymerization, the former monomer gave the cellulose-type (1 → 4)-β-D-ribopyranan [36,37], the latter gave a polymer with mixed structures consisting of 1,4-β ribopyranosidic and 1,5-α ribofuranosidic structures [41]. A polymerization temperature of 0°C worked effectively to give 1,4-β pyranosidic structure. The stereoregularity of the copolymers decreased with an increase of the molar ratio of the silylated monomer in the feed. Selective desilylation and subsequent glycosylation with D- or L-glucose ethylorthoacetate gave branched ribopyranans after deprotection [95] (Scheme 32).

Synthesis of branched ribofuranans was carried out by the ring-opening copolymerization in the various ratios of 1,4-anhydro-2,3-di-*O*-benzyl- and -2,3-di-*O-tert*-butyldimethylsilyl-α-D-ribopyranoses with $BF_3{\cdot}OEt_2$ in CH_2Cl_2. Selective removal of the silylated groups gave partially benzylated (1 → 5)-α-D-ribofuranan, which was reacted with D-mannose, D-glucose, and L-glucose orthoesters to afford branched ribofuranans after deprotection having branches at the C2 and/or C3 positions on the ribofuranan main chain. After sulfation, the branched ribopyranans and ribofuranans led to sulfonated branched polysaccharides with strong anti-HIV activity [96] (Scheme 33).



Scheme 33.

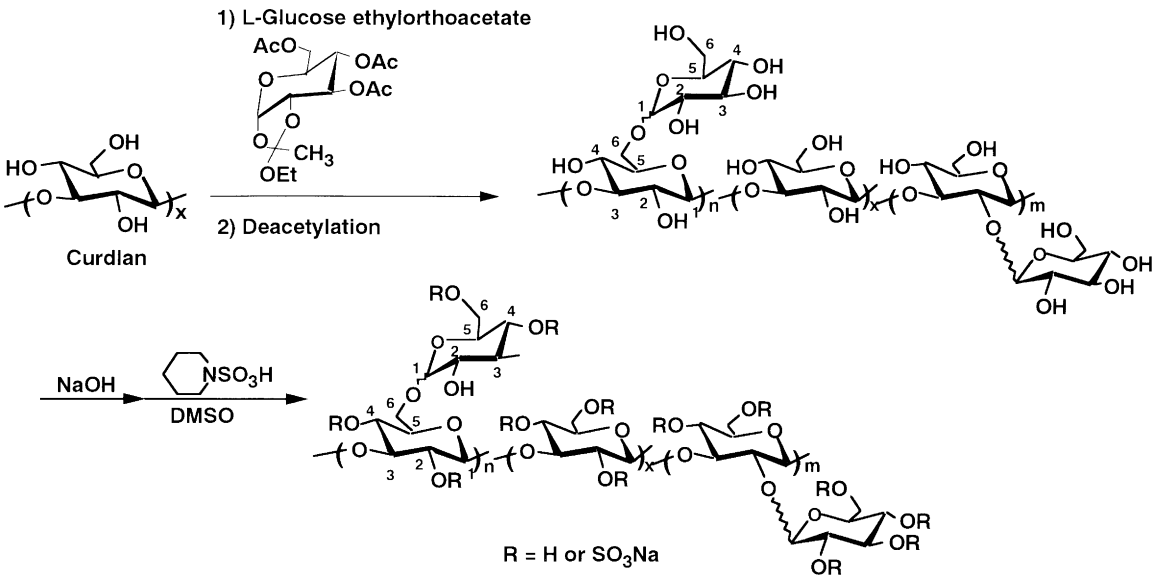
3.3. Glycosylation to natural linear polysaccharides

In general, natural polysaccharides have branched structures, which induce the specific biological properties such as anti-tumor activity and solubility in water. Since the natural branched polysaccharides have complex structures, it is difficult to elucidate the relationship between the structure and biological activities. Synthesis of branched polysaccharides by glycosylation to natural linear polysaccharides is a facile method to prepare branched polysaccharides having high molecular weights. However, natural linear polysaccharides, in general, have low solubility and reactivity.

Cellulose acetate having β-D-glucose branches was synthesized by condensation of 3,4,6-tri-*O*-acetyl-(1,2-*O*-ethylorthoacetyl)-α-D-glucopyranose with activated cellulose acetates (DS = 2) in boiling chlorobenzene in the presence of a catalytic amount of 2,6-dimethylpyridinium perchlorate [97]. The branched cellulose acetates with more than 30 mol% of branching were converted into water-soluble polysaccharides. The branching occurred preferentially at the C6 positions by β-glycosidic linkage. Cellulose did not react with the acetyl glucose orthoester. The position and proportion of branches were determined by methylation analysis and ^{13}C NMR spectroscopy [98].

By the same procedures, curdlan, a linear 1,3-β-D-glucan, and curdlan acetate were reacted with the orthoacetate to give branched curdlan and curdlan acetate [99], whose polysaccharides with more than 60 and 30 mol% of branches, respectively, were completely soluble in water. The anti-tumor activity of the synthesized branched-cellulose and curdlan was investigated.

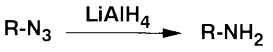
We synthesized L-glycosyl-branched curdlan for increasing anti-HIV activity described later. Natural and non-natural monosaccharides such as D- and L-glucoses and D- and L-mannoses were reacted with curdlan to form branched curdlan, respectively, which led to sulfonated branched curdlans by sulfonation and then anti-HIV and blood anti-coagulant activity were examined [100] (Scheme 34).



Scheme 34.

4. Synthesis of amino-polysaccharides

Since the azido group is a precursor of the amino group and is stable to acid, anhydro-sugar monomers having azido groups were synthesized and polymerized to give amino-polysaccharides. The azido group was converted easily to an amino group by the reduction of lithium aluminum anhydride (Scheme 35). Natural amino-polysaccharides and their sulfonated derivatives had specific biological activities such as strong blood anti-coagulant activities for heparin, and anti-microbial and wound healing effects for chitin. Therefore, in order to know the structure–activity relationship, the synthesis of amino-polysaccharides with apparent structure is an important and interesting study.



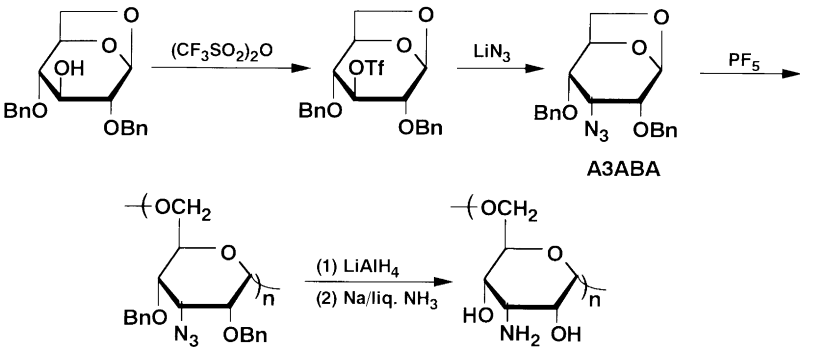
Scheme 35.

4.1. Hexopyranan-type amino-polysaccharides

In 1983, Uryu and Hatanaka reported for the first time that the synthesis of amino-group-containing (1 → 6)-α-D-glucan derivatives by ring-opening polymerization of 1,6-anhydro-α-D-glucopyranose with an azido group at the C2, C3, or C4 position [101,102]. Among the anhydro azido monomers, 3-*O*-azido monomer was polymerized by a Lewis acid catalyst to give 3-amino dextran having an amino group at the C3 position on the repeated glucose unit after the reduction of the azido group into an amino group. The molecular weight of the azido polymer was $\bar{M}_n = 5.5 \times 10^3$. However, the C2- and C4-azido monomers were not polymerized.

For the improvement of the low polymerizability of the 2-azido-monomer, a new amino protecting monomer, 1,6-anhydro-2-deoxy-3,4-di-*O*-benzyl-2-phthalimino-β-D-glucopyranose was synthesized from 1,6-anhydro-β-D-mannopyranose in several steps and polymerized to yield 1,6-β-linked glucosamine oligomers with $\overline{DP}_n = 5$–7, which 1,6-β formation was presumably due to the steric hindrance by the 2-phthalimido substituent. After deprotection and then *N*-acetylation, 2-acetamido-2-deoxy-(1 → 6)-β-D-glucopyranan was obtained [103].

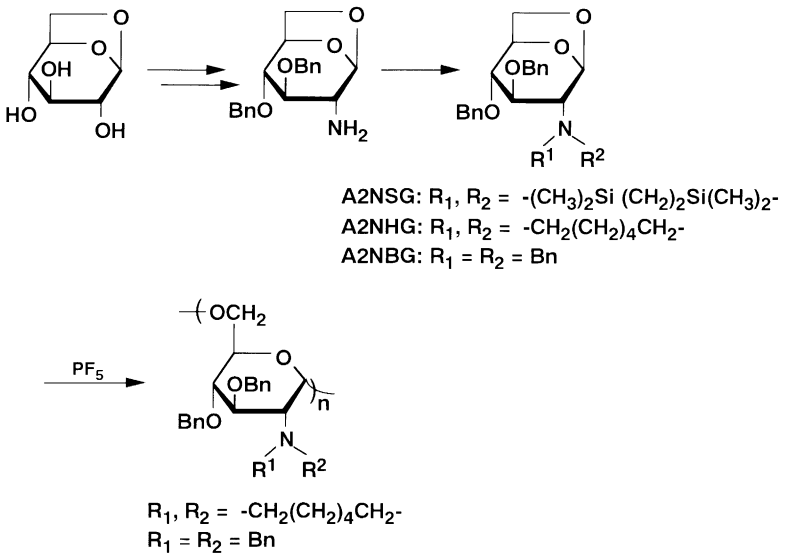
Thus, we synthesized a new monomer, benzylated 1,6-anhydro-3-azido-3-deoxy-β-D-allopyranose and attempted to polymerize it into amino-polysaccharides with 1,6-α-linked allopyranosidic structure [104]. The ring-opening polymerization of 1,6-anhydro-3-azido-2,4-di-*O*-benzyl-3-deoxy-β-D-allopyranose (A3ABA) was performed with a catalyst to give a stereoregular (1 → 6)-α-D-allopyranan derivative having an azido group at the C3 position. The molecular weight was high, $\bar{M}_n = 24.0 \times 10^3$. The reduction of the azido groups into amino groups by lithium aluminum hydride and the subsequent removal of the benzyl groups to hydroxyl groups afforded 3-amino-3-deoxy-(1 → 6)-α-D-allopyranan in a good yield. In addition, in order to synthesize amino-polysaccharides having different proportions of amino sugar unit on the polysaccharide backbone, the copolymerization of A3ABA with 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (LGTBE) in the various monomer feeds was carried out to give the corresponding copolymers. The results of calculation of the monomer reactivity ratio, $r_{A3ABA} = 0.66$ and $r_{LGTBE} = 1.67$, suggest that the A3ABA unit was distributed randomly on the polymer backbone. New hetero-polysaccharides consisting of glucopyranose and 3-amino-allopyranose units were obtained



Scheme 36.

after the reduction of azido groups into amino groups and benzyl groups into hydroxyl groups (Scheme 36).

New 1,6-anhydro-glucosamine monomers having amino protecting groups at the C2 position, 1,6-anhydro-3,4-di-*O*-benzyl-2-(*N*,*N*-cyclo(1,2-bis(dimethylsilyl)ethyl-amino)-2-deoxy-β-D-glucopyranose (A2NSG), 1,6-anhydro-3,4-di-*O*-benzyl-2-hexamethyleneimino-2-deoxy-β-D-glucopyranose (A2NHG) and 1,6-anhydro-3,4-di-*O*-benzyl-2-(*N*,*N*-dibenzylamino)-2-deoxy-β-D-glucopyranose (A2NBG) were synthesized in order to investigate the effects of 2-amino groups on cationic ring-opening polymerizations [105]. A2NSG monomer was polymerized with PF_5 and $SbCl_5$ catalysts to give 1,6-α stereoregular polymers with the molecular weights of $\bar{M}_n = 4.1 \times 10^3$ and 3.4×10^3, respectively. A2NHG monomer had low polymerizability and A2NBG was not polymerized (Scheme 37). The low polymerizability of the substituted amino group containing monomers might be attributed to (1) high nucleophilicity of the nitrogen in the substituted amino groups, (2) high electron density of the nitrogen bonded to silicones



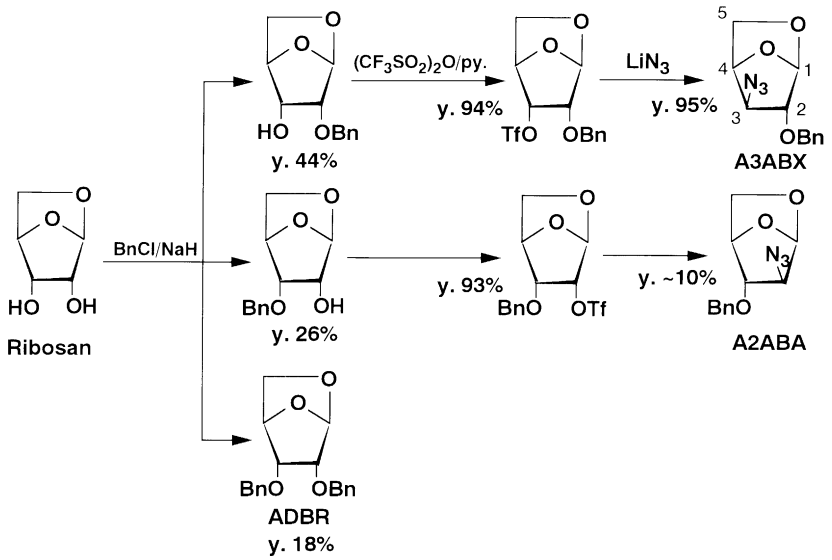
Scheme 37.

having low electronegativity in the case of A2NSG, and (3) bulkiness of the wholly substituted amino groups. According to (1) and (2), Lewis acid catalysts might tend to coordinate with the N2 nitrogen in addition to the O6 oxygen, and thereby the polymerizability decreased. As for (3), Kobayashi and Schuerch reported that a bulky axial substituent at the C2 position has a great effect on the polymerizability of 1,6-anhydro-sugars [44]. Therefore, low conversions of A2NHG having the bulky cyclic hexamethylene group at the C2 position might be owing to the difficult approach of the monomer to a propagating end.

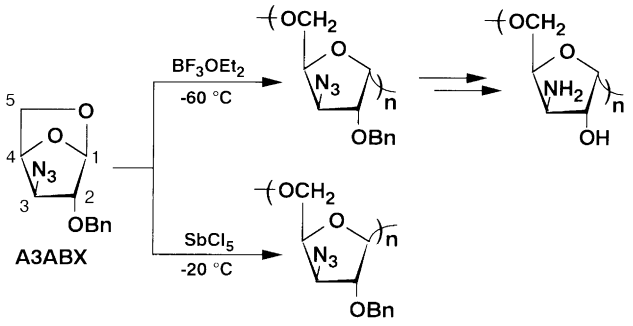
4.2. Pentofuranan-type amino-polysaccharides

Most amino sugars are presented in nature as hexoses such as glucosamine and galactosamine or their derivatives in the polysaccharide or oligosaccharide chains, and pentose-type amino sugars are rare. There are several reports on the chemical synthesis of amino-polysaccharides consisting of hexoses as mentioned in Section 4.1. There had been no reports on the synthesis of pentofuranan-type amino-polysaccharides until 1996. Since 1,4-anhydro-pentose monomers have high polymerizability, we expected new pentofuranosidic amino polysaccharides having high molecular weights. In order to know the structural information of amino polysaccharides on their biological activities, the synthesis of pentose-type amino-polysaccharides having defined configurational structures is important.

Initially, we synthesized a new xylose-type azido monomer, 1,4-anhydro-3-azido-2-*O*-benzyl-3-deoxy-α-D-xylopyranose (A3ABX) from D-ribose and then the monomer was polymerized to give 3-amino-3-deoxy-(1 → 5)-α-D-xylofuranan [47]. The monomer was synthesized by benzylation of 1,4-anhydro-α-D-ribopyranose at the C2 hydroxyl group and subsequent trifluoromethylsulfonylation at the C3 position, followed by S_N2 replacement of the trifluoromethanesulfonyl group into an azido group with lithium azide. We also synthesized a 2-azido monomer, 1,4-anhydro-2-azido-3-*O*-benzyl-2-deoxy-α-D-arabinopyranose (A2ABA) from D-ribose. However, the yield of the 2-azido monomer was low. Therefore, we attempted to polymerize the 3-azido monomer (Schemes 38 and 39).



Scheme 38.



Scheme 39.

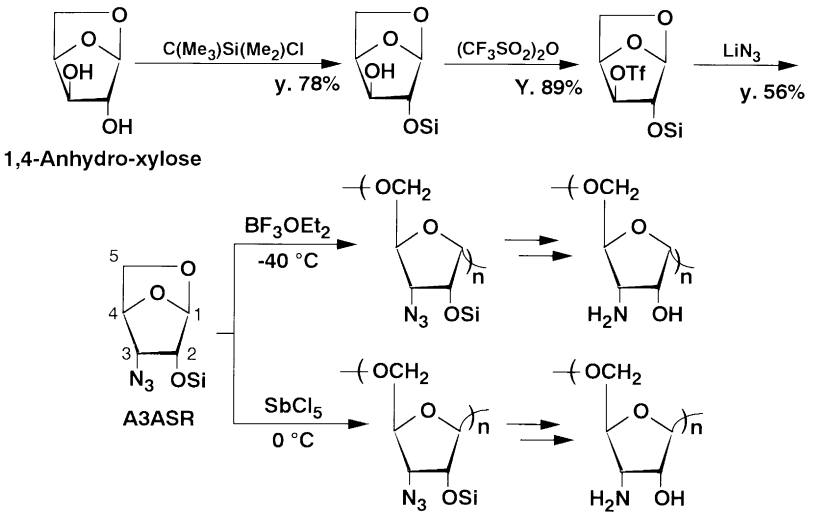
The ring-opening polymerization of A3ABX was carried out with Lewis acid catalysts at different temperatures between −60 and −20°C (Table 1). Boron trifluoride etherate catalyst gave a polymer with a positive specific rotation and with a single C1 absorption at 99 ppm in the ^{13}C NMR spectrum, indicating that the polymer had a stereoregular 1,5-α furanosidic structure. When the polymerization was performed with antimony pentachloride at −60°C, the polymer obtained had a negative specific rotation and the C1 absorption shifted downfield to around 107 ppm, suggesting that the polymer might be composed of a 1,5-β furanosidic unit. Reduction of the azido group to an amino group and benzyl group to hydroxyl group gave 3-amino-3-deoxy-(1 → 5)-α-D-xylofuranan.

We also synthesized a new ribose-type azido monomer from D-xylose and then polymerized it [106]. The monomer, 1,4-anhydro-3-azido-2-*O*-benzyl-3-deoxy-α-D-ribopyranose (A3ASR), was synthesized from 1,4-anhydro-α-D-xylopyranose by three steps comprising Walden inversion at the C3 position into ribose configuration, that is, selective 2-*O*-silylation by *tert*-butyldimethylsilyl chloride, 3-*O*-triflation by trifluoromethanesulfonic anhydride, and subsequent S_N2 replacement with lithium azido afforded A3ASR as colorless needle-like crystals after recrystallization. The ring-opening polymerization by Lewis acid catalysts at −60 or −40°C gave a stereoregular 3-azido-3-deoxy-2-*O*-*tert*-butyldimethylsilyl-(1 → 5)-α-D-ribofuranan having specific rotations of +246 to +271° and having number-average molecular weights of

Table 1
Ring-opening polymerization of A3ABX

No.[a]	Catalyst	Mol%	Temperature (°C)	Time (h)	Yield (%)	M_n[b] × 10^4	$[\alpha]_D^{25}$[c]	α-content[d] (%)
1	BF_3OEt_2	5	−40	5	48	2.3	+16.8	100
2	PF_5	5	−20	0.25	86	3.9	+17.7	100
3	PF_5	5	−40	0.25	91	2.9	+14.0	100
4	PF_5	5	−60	0.25	72	4.4	+21.2	100
5	$SbCl_5$	3	−20	3	74	1.8	+2.8	45
6	$SbCl_5$	3	−40	3	54	1.1	+1.3	25
7	$SbCl_5$	3	−60	2.5	42	3.2	−4.2	10

[a] Monomer: 0.24–0.29 g; solvent: CH_2Cl_2, 0.5 ml.
[b] Determined by GPC.
[c] Measured in $CHCl_3$ (*c*, 1%).
[d] From ^{13}C NMR spectrum.



Scheme 40.

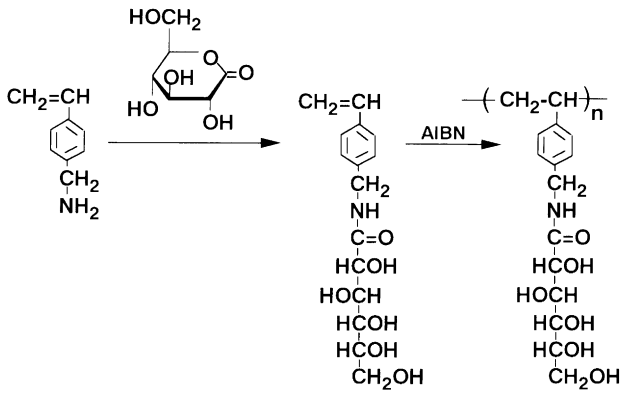
18.7×10^3–25×10^3. When the polymerization was carried out with antimony pentachloride at 0°C, the resulting polymer provided a negative specific rotation of −6° and the C1 signal in the ^{13}C NMR spectrum shifted downfield to 107.5 ppm, suggesting that the polymer might consist of a 1,5-β furanosidic unit. The reduction of the azido group of the 1,5-α and 1,5-β furanosidic polymers into an amino group and subsequent desilylation gave 3-amino-3-deoxy-(1 → 5)-α- and -β-D-ribofuranans, respectively (Scheme 40; Fig. 9).

An acetamido group containing xylofuranan, 3-acetamido-3-deoxy-(1 → 5)-α-D-xylofuranan was prepared from 1,4-anhydro-3-azido-2-*O*-*tert*-butyldimethylsilyl-3-deoxy-α-D-xylopyranose (A3ASX). The xylose-type azido monomer was synthesized from 1,4-anhydro-2-*O*-*tert*-butyldimethylsilyl-α-D-ribofuranose according to the same procedure as that of the A3ABX monomer [107]. The ring-opening polymerization of the A3ASX monomer with $BF_3{\cdot}OEt_2$ at −20°C gave stereoregular 3-azido-2-*O*-*tert*-butyldimethylsilyl-3-deoxy-(1 → 5)-α-D-xylofuranan having a high molecular weight of $\bar{M}_n = 178 \times 10^3$ in 92% yield. After conversion of the azido group to an acetamido group and then desilylation, 3-acetamido-3-deoxy-(1 → 5)-α-D-xylofuranan was obtained. Ribose-type acetamide-containing polysaccharide, 3-acetamido-3-deoxy-(1 → 5)-α-D-ribofuranan was synthesized by the ring-opening polymerization of A3ASR [108].

5. Synthetic polymers having pendant poly- or oligosaccharides

Synthetic polymers having pendant oligosaccharide chains are of interest for model compounds to elucidate the specific biological activities of complex natural poly- and oligosaccharides in proteoglycans, glycoproteins, and glycolipids on the surface of cells as well as for artificial biological materials. A number of reports have been published on the synthesis of this type of polymer with defined structure.

The radical polymerization of styrene derivatives, which were obtained by the coupling of oligosaccharide lactones with *p*-vinylbenzylamine, gave polystyrene derivatives having pendant

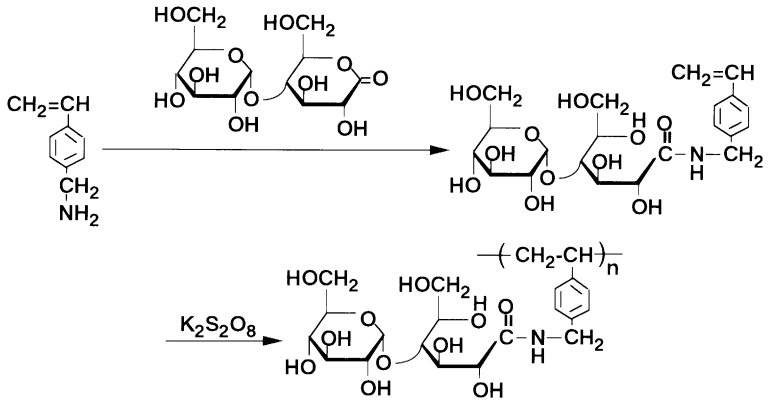


Scheme 41.

oligosaccharides [109]. *N-p*-Vinyl-D-glucosamide was prepared by a coupling reaction between D-glucono-1,5-lactone and *p*-vinylbenzylamine [110]. It is not necessary to protect the hydroxyl groups in sugar lactones. The reaction procedure is simple and involves only the mixing of the two reactants in refluxing methanol to give styrene derivatives in a quantitative yield (Scheme 41). The sugar lactone was prepared easily by oxidation of a reducing sugar. The radical polymerization with azobis(isobutyronitrile) (AIBN) in DMSO yielded a water-soluble polymer, which was found to have a strong affinity for methyl orange and magnesium 1-amino-8-naphtalenesulfonate in water.

Polystyrene derivatives with maltose, lactose, and maltotriose on each repeating unit were prepared by the same procedures as above [109,111]. The lactose-carrying polystyrene was a useful surface material for a liver cell (hepatocyte) culture (Scheme 42 — synthesis and polymerization of *N-p*-vinylbenzyl-[*O*-α-D-glucopyranosyl-(1 → 4)]-D-gluconamide).

A vinyl monomer having a β-linked *N*-acetyllactosamine moiety, a major component of oligosaccharide chains of glycoproteins, on each repeating unit was synthesized by a chemo-enzymatic synthesis, using a β-galactosidase as catalyst, from *p*-nitrophenyl *N*-acetyl-β-D-glucosamine and lactose. The



Scheme 42.

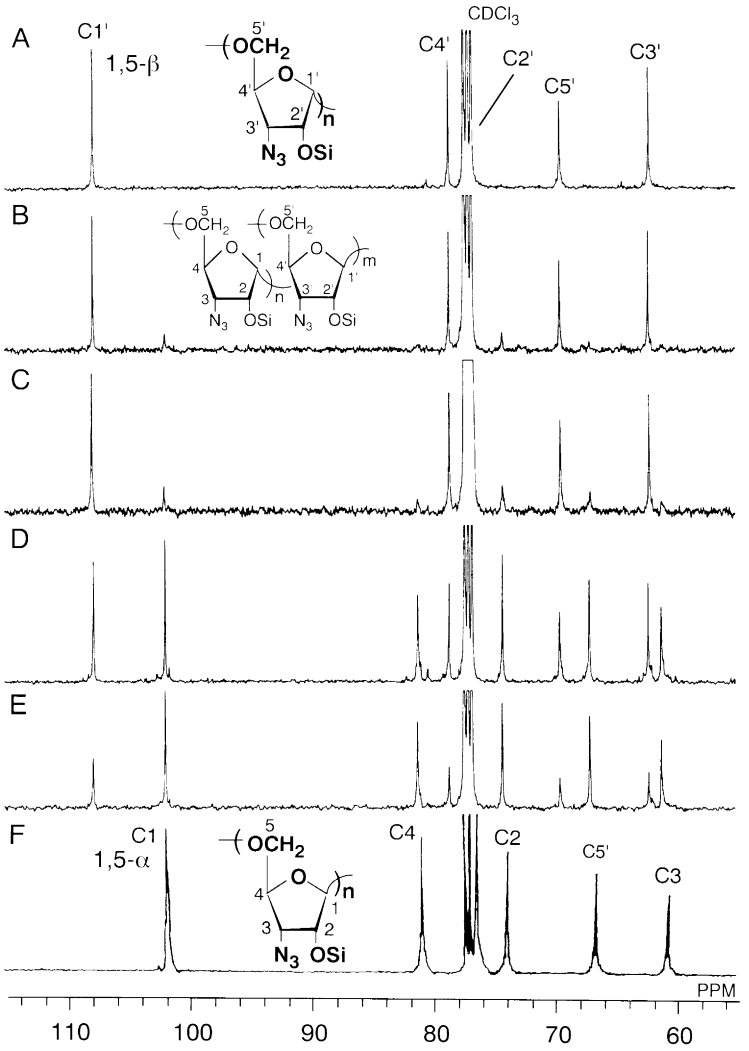
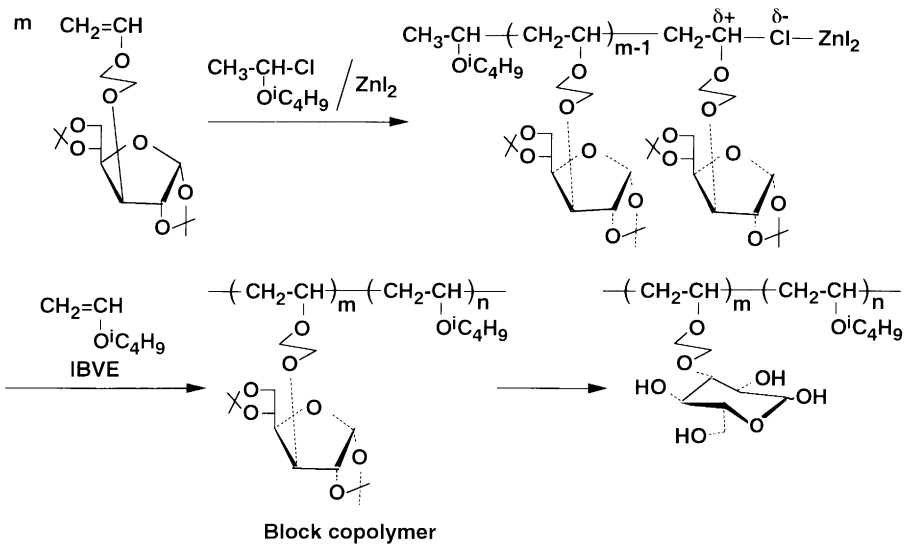


Fig. 9. ^{13}C NMR spectra of poly(A3ASR)s prepared by $SbCl_5$ at (A) 0, (B) −20, (C) −40, (D) −60, (E) −78°C, and (F) 3-azido-2-*O*-*tert*-butyldimethylsilyl-3-deoxy-(1 → 5)-α-D-ribofuranan prepared by BF_3OEt_2 at −40°C ($CDCl_3$ as solvent).

nitro group was reduced to an amino group, which was then allowed to react with either acryoyl chloride or acrylic acid to give styrene derivatives. The resulting *p*-acryloylaminophenyl *N*-acetyl-β-lactosaminide was polymerized with AIBN in DMSO to give an *N*-acetyl-β-D-lactosamine-carrying polyacrylamide derivative having $\bar{M}_n = 3.2 \times 10^5$.

Acrylamide derivatives having long alkylene spacers were synthesized by the regioselective esterification of methyl glucopyranoside and 3-*O*-methylated glucopyranose with 11-methacryloylaminoundecanoic acid in the presence of a lipase from *Candida antarctica*. These monomers were polymerized radically with AIBN [112].

A D-glucosamine-containing vinyl ether monomer was prepared by two steps, that is, the glycosylation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimide-β-D-glucopyranosyl bromide was performed with



Scheme 43.

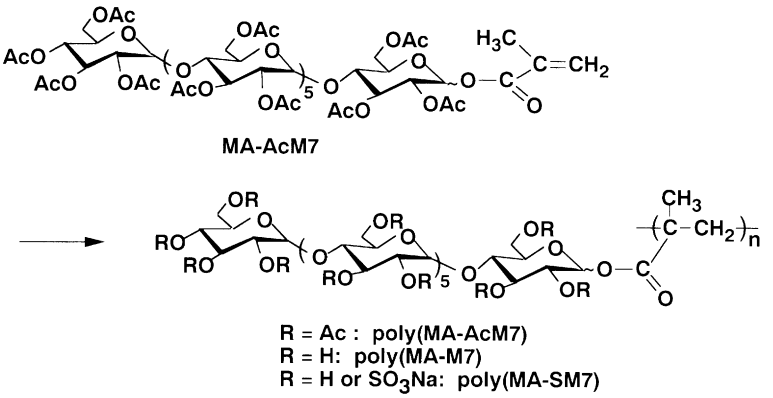
ethylene glycol to give 1-*O*-(hydroxyl)ethyl-3,4,6-tri-*O*-acetyl-2-deoxy-phthalimide-β-D-glucopyranoside, which was carried out through transetherification with 2-chloroethyl vinyl ether in the presence of silver trifluoromethanesulfonate to give the vinyl ether monomer. The living polymerization was attempted by using a cationic initiating system, an adduct of trifluoroacetic acid (TFA) and isobutyl vinyl ether (TFA/$EtAlCl_2$ initiating system), in toluene at 0°C to give a polymer with narrow molecular weight distribution of $\bar{M}_w/\bar{M}_n = 1.1$ and $\bar{M}_n = 2.4 \times 10^4$. The deprotection of the resulting polymer was performed with hydrazine monohydrate to afford the corresponding glycopolymers bearing pendant D-glucosamine residues [113,114]. The sequential living polymerization of a glucose-carrying vinyl ether, 3-*O*-(vinyloxy)ethyl-1,2:5,6-di-*O*-isobutylidene-D-glucofuranose, with isobutyl vinyl ether to give block copolymers (Scheme 43). After deprotection, amphiphilic block copolymers were obtained [115].

Non-ionic hydrogels bearing glucoside residues were synthesized by free-radical copolymerization of glucosylethyl methacrylate (GEMA) and *N*,*N*′-methylene-bisacrylamide (BisA) with ammonium peroxodisulfate and *N*,*N*,*N*′,*N*′-tetramethyl-ethylenediamine as the initiator at 0°C for 2 h. Copolymerization of GEMA, sodium acrylate, and BisA gave anionic hydrogels. The non-ionic and anionic gels absorbed 30 and 100 times as much water as their own weight, respectively [116].

In order to know the relationship between structure and biological activities of polymethacrylates having pendant oligosaccharide chains, a new acetylated 1-*O*-methacryloyl maltoheptaoside was polymerized with AIBN to give polymethacrylates having a pendant acetylated maltoheptaose in every repeating unit. After deacetylation, the polymethacrylates were sulfonated to examine the anti-HIV activity as described in Section 6 (Scheme 44).

6. Biological activity of synthetic and natural polysaccharides

Acquired immunodeficiency syndrome (AIDS) is a disease caused by a retrovirus, HIV, which



Scheme 44.

destroys the body's immune system. Our body tends to be vulnerable to a number of infections, which are very unusual, when the immune system is working normally. Since the first discovery of AIDS in 1981 and the isolation of the causative virus, HIV, in 1983, many anti-viral drugs have been developed and attempted for the prevention and therapy of AIDS. However, there is no medicine to completely treat AIDS. An AIDS vaccine has been developed also; however, it is difficult to make AIDS vaccines because of the frequent mutation of the AIDS virus. Presently, by consideration of the replication cycle of the AIDS virus, some hopeful and approval medicines such as 2,3-dideoxyazidothimidine (AZT), 2,3-dideoxyinocine (ddI), 2,3-dideoxycytocine (ddC), and some protease inhibitors have been used to cure AIDS.

The first report on the polysaccharides having anti-virus activity, in which polysaccharides extracted from sea weeds inhibited the infection of influenza B and mumps viruses, appeared in 1958 [117]. Inhibitory effects on the herpes simplex virus were also reported in 1964 [118,119]. In 1987, dextran sulfonate was found to have inhibitory effects on the reverse transcriptase (RT) of retrovirus and to possess possibly an anti-HIV activity [120]. Dextran sulfonate has potent anti-HIV activity in vitro. However, in 1989, it was reported that no anti-HIV activity was observed by oral administration of dextran sulfonate [121]. On the other hand, the inhibitory effects of sulfonated polysaccharides were demonstrated in 1987, where an aqueous extract from sea red alga, *Schizymenia pacifica*, inhibited the activity of RT from both the avian myeloblastosis virus and the Rauscher murine leukemia virus [5]. It was found that the sea alga extract, which was a sulfonated polysaccharide, inhibited the RT of HIV and replication in vitro. Some natural sulfonated polysaccharides such as condroitin, dermatan, heparin, and keratan sulfonates also inhibited the RT of the avian myeloblastosis virus [6]. The anti-HIV activity of sulfonated polysaccharides is assumed to inhibit the binding of HIV to the T cell in the first stage of infection [122].

Another important biological activity of sulfonated polysaccharides is the blood anti-coagulant activity [123]. Heparin is a naturally occurring sulfonated mucopolysaccharide having potent anti-coagulant activity and is used for clinical purposes. Heparin combined initially to anti-thrombin III (AT-III) and then the complex inhibits the activity of thrombin, a blood coagulant factor, to cause strong blood anti-coagulation [124].

In this section, the synthesis and anti-AIDS virus and blood anti-coagulant activities of sulfated

polysaccharides obtained by sulfation of artificial ribofuranans, ribopyranans, dextrans, and naturally occurring curdlan are described. In addition, the structure–activity relationship of sulfated polysaccharides will be provided.

6.1. Anti-HIV activity and blood anti-coagulant activity

As a preliminary, we found the anti-HIV activity of synthetic sulfonated polysaccharides in 1987. The anti-HIV activity was tested in vitro by using an MT-4 cell which is an HIV-sensitive cell line and dies 6 days after HIV-infection. Dextran, ribofuranan, and xylofuranan sulfonates completely prevented HIV-induced cytopathic effects at concentrations less than 10 μg/ml. The anti-HIV activity of these sulfonated polysaccharides was confirmed by measuring the HIV-specific antigen expression in infected MT-4 cells. In the cocultures with MOLT-4 and MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells, the formation of multinucleated cells was completely inhibited in the presence of 100 μg/ml of these sulfonated polysaccharides [125]. Sulfonated polysaccharides having negatively charged sulfonate groups interact with positive amino acid residues in the surface glycoprotein gp120 of HIV to prevent HIV from attaching to human T-lymphocytes.

The anti-HIV activity was examined by the indirect immunofluorescence (IF) method [125] and by the MTT method [126], respectively. The 50% effective concentration, EC_{50}, was calculated by the dose of sulfonated polysaccharides achieving 50% protection of HIV infection to the MT-4 cell. The cytotoxicity (CC_{50}) was determined by the 50% cytotoxic concentration on the MT-4 cell. The blood anti-coagulant activity was determined by using bovine plasma according to the United States Pharmacopoeia [127]. The blood anti-coagulant activity is an important biological activity of sulfonated polysaccharides. However, the anti-coagulant activity would be a side effect for anti-HIV activity, because high anti-coagulant sulfonated polysaccharides strongly interact with the blood coagulation proteins in plasma and selective interaction to HIV may not accrue.

6.1.1. Sulfonated curdlan

Curdlan, a natural linear 1,3-β-D-glucan, was sulfonated with piperidine *N*-sulfonic acid in DMSO to give curdlan sulfonates with several molecular weights and sulfur contents [128] (Fig. 10). Curdlan sulfonates with a sulfur content of 14.4% completely inhibited the infection of HIV in the drug concentration as low as 3.3 μg/ml and had low cytotoxicity. Table 2 summarizes the minimum effective concentration of curdlan sulfonates on HIV infection to the MT-4 cell. Low sulfur content curdlan sulfonates showed a low anti-HIV activity even though they had high molecular weights (Nos. 1 and 2). However, curdlan sulfonates having high sulfur contents exhibited high anti-HIV activity of 3.3 μg/ml. The anti-coagulant activity was less than 10 unit/mg compared with standard dextran sulfonate

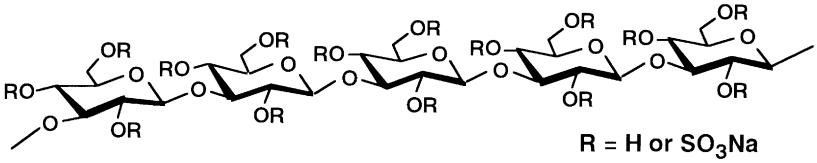


Fig. 10. Structure of curdlan sulfonate.

Table 2
Anti-HIV activity of curdlan sulfonate (minimum effective concentration of curdlan sulfate on complete inhibition of HIV infection)

No.	S content (%)	DS	$\bar{M}_n \times 10^4$	$[\alpha]_D^{25}$ (°)	Anti-HIV activity
1	5.6	0.35			No effect
2	8.9	0.8	6.9		1000
3	12.2	1.1	8.1	−1.7	10
4	12.1	1.1	11.8	−3.8	3.3
5	12.5	1.3	15.7	−2.3	3.3
6	13.6	1.4	3.4	−0.8	3.3
7	14.1	1.6	2.1	−1.9	3.3
8	14.4	1.6	4.6	+0.1	3.3
9	14.7	1.6	2.0	−1.5	3.3

(NC-1032, 20.6 unit/mg), suggested that curdlan sulfonates were preferred for an anti-HIV drug because of low cytotoxicity and low anti-coagulant activity.

The high-resolution NMR analysis including two-dimensional experiments revealed that for a curdlan sulfonate with the DS of 1.6 per glucose unit, the sulfonate group was introduced into the C6, C2, and C4 positions of the glucose unit in the proportions of 1, 0.5, and 0.1, respectively.

In order to evaluate the inhibitory activity of curdlan sulfonates on HIV, therapeutic availability in preliminaries was carried out [129]. The cytotoxicity on an uninfected MT-4 cell has not been observed at a concentration of up to 5000 μg/ml. Therefore, the toxicity of curdlan sulfonates in detail was preliminarily examined employing animal models. LD_{50} of curdlan sulfonate in intravenous injection was found at around 2000 mg/kg employing mice and rats, and neither death nor hemorrhage was observed in consecutive administration of curdlan sulfonate for 2 weeks at doses of 50 mg/kg/day in Sprague-Dawley rats. The half-life of curdlan sulfonate in plasma was found to be different depending on its molecular weight, that is, it was 60 and 180 min for curdlan sulfonate having $\bar{M}_n = 70 \times 10^4$ and 170×10^4, respectively. In order to maintain an effective concentration of 5 mg/ml in blood to inhibit the HIV-1 infection, the dosage for continuous infusion was calculated as 6.5–19.5 mg/h in humans depending on the molecular weights of curdlan sulfonate. The total dosage required per day was calculated as approximately 160–470 mg/day/man, that is, 3.2–9.4 mg/kg/day. These suggested dosages for intravenous injection of curdlan sulfonate to humans should be carefully examined by further toxicological analysis. In addition, the activity of curdlan sulfonate against HIV-1 was assessed in curdlan sulfonate-depleted cell culture following incubation with MT-4/HIV-1, MT-4 cells and curdlan sulfonate for different periods in vitro. The HIV-1 cellular infectivity completely disappeared in curdlan sulfonate-depleted cell culture after incubation for 168 h at a curdlan sulfonate concentration of 5 mg/ml or more. Thus, the cells were never infected after incubation with curdlan sulfonate.

The phase I/II test was carried out in the USA, because the favorable toxicological profile of curdlan sulfonate in animals suggested clinical trials [130]. In monkey studies, no prolongation of the activated partially thromboplastin time (APTT) was seen with intravenous doses of 2 mg/kg, corresponding to blood levels of about 35 μg/ml. Doses of 0.014, 0.14, 0.42, 1.42, 2.84, and 4.26 mg/kg (which worked out to 1, 10, 30, 100, 200, and 300 mg/body, respectively) were administered to HIV-positive patients at each dose level for 4 h intravenously. APTTs were measured hourly. Based on APTT data, the maximum safe dose was approximately 2.84 mg/kg or 200 mg/70 kg patient. There were no clinical side effects at any dose tested in

this study. Fig. 11 shows the effect of curdlan sulfonate on CD4 levels with increasing dose from 1 mg to 300 mg/70 kg. HIV infected T cells through a virus surface glycoprotein gp120 with CD4 and the chemokine receptor CXCR4 on T cells or CCR5 on macrophages [131]. Thus, the CD4 level in blood is an index for the progress of AIDS. The bars show the increase or decrease of CD4 levels from the baseline after the 4-h infusion. It was observed that single doses of curdlan sulfonate produced marked and dose-related increases in CD4 lymphocytes in HIV-infected patients. HIV will only survive in the blood stream for 3 or 4 days without infection to CD4 positive T cells. Thus, if the binding can be prevented for more than 4 days after infection with HIV, we may be able to reduce or eliminate it ineffectively. From these preliminary clinical results, curdlan sulfonate is safe for multiple dosing with the monitoring of APTT.

To maintain the concentration of curdlan sulfonate in the blood, we synthesized branched curdlan sulfonates and measured the APTT of the blood in vivo. Natural and non-natural sugars such as D- and L-glucoses and -mannoses were reacted with curdlan to form branched curdlan (Scheme 34), respectively. Table 3 summarizes the results of anti-HIV activity in vitro and APTT by using mice. These branched curdlan sulfonates inhibited the cytopathic effect caused by HIV infection at low concentrations. The EC_{50} ranged from 0.3 to 1.2 μg/ml, suggesting that the branched curdlan sulfonates had potent anti-HIV activity. The cytotoxicity was low, because these sulfonates did not inhibit the cell growth at the concentration of more than 1000 μg/ml. The anti-coagulant activity of D- and L-glycosyl-branched curdlan sulfonates was calculated from the APTT by using a linear curdlan sulfonate and heparin as references. It is noteworthy that the D- and L-glucose branched curdlan sulfonates had the anti-coagulant activity of 18.5 and 17.2 unit/mg, respectively, which are almost equivalent to that of the linear curdlan sulfonate of 19.0 unit/mg. The anti-coagulant activity was roughly one-tenth of that of heparin (150 unit/mg), suggesting that the rigid rod-like structure of curdlan sulfonate was related to the weak interaction with the coagulant enzymes in plasma. In addition, the time dependence on the concentration of D- and L-glucose branched curdlan sulfonates in mice in vivo are shown in Fig. 12. The branched sulfonates were intravenously injected into mice at a dose of 10 mg/kg, that is, the initial concentration in the blood

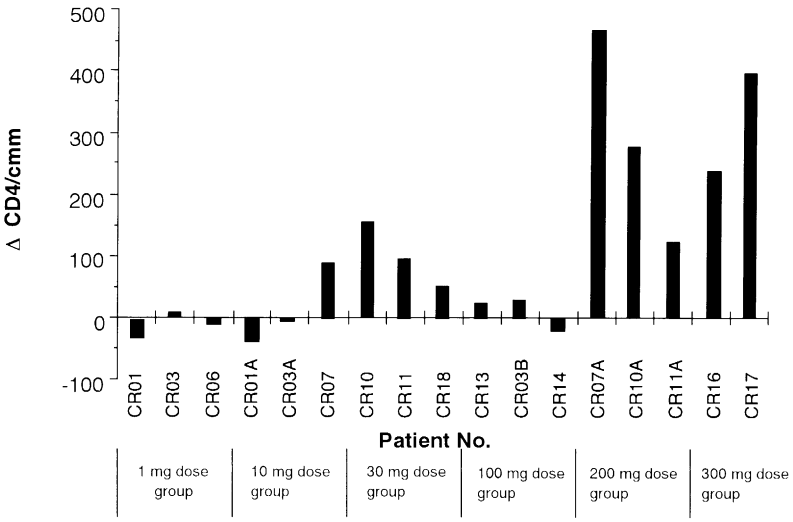


Fig. 11. Effect of curdlan sulfonate on CD4 levels with increasing dose from 1 mg to 300 mg/70 kg. The bars show the increase of CD4 levels from baseline after the 4-h infusion.

Table 3
Anti-AIDS virus activity of branched curdlan sulfates

No.	Curdlan sulfate[a] (branch)	S content (%)	$\bar{M}_n \times 10^4$	$[\alpha]_D^{25}$ (°)	EC_{50}[b] (μg/ml)	CC_{50}[c] (μg/ml)	AA[d] (unit/mg)
1	D-GCS1 (39%)	13.2	4.2	+10.0	0.9	>1000	18.5
2	D-GCS2 (39%)	14.4	3.6	+10.5	0.3	>1000	
3	L-GCS1 (35%)	13.2	4.7	+0.9	1.2	>1000	17.2
4	D-MCS1 (19%)	15.1	1.7	+12.5	0.6	>1000	
5	L-MCS1 (17%)	15.2	1.2	+10.9	0.5	>1000	
6	CS	14.1	7.9		0.43	>1000	
7	DS	18	0.7		0.91	>1000	
8	AZT (mM)				0.0019	6.43	

[a] D-GCS: D-glucose-branched curdlan sulfate; L-GCS: L-glucose-branched curdlan sulfate; D-MCS: D-mannose-branched curdlan sulfate; L-MCS: L-mannose-branched curdlan sulfate; CS: curdlan sulfate; DS: Dextran sulfate.
[b] 50% Effective concentration.
[c] 50% Cytotoxic concentration.
[d] APTT: activated partially thromboplastin time.

was calculated to be 250 μg/ml. The blood samples were withdrawn 20, 60, and 180 min after injection, and then the concentrations of the sulfonates in plasma were evaluated from the APTT. The concentration of the sulfonates was one-third of the initial concentration 3 h after injection. However, there was almost no significant difference in the behaviors in the blood between D- and L-glycosyl-branched curdlans and also between the linear and branched curdlans. The disappearance of curdlan sulfonates from the blood was due to absorption mainly in such tissues as the liver, bone marrow, kidney, and lymph node without degradation for 10 days [132]. This might be preferred to protect from the infection of HIV because HIV is found often in the first step of infection in the lymph node.

Two kinds of HIV are known, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 is an ordinary HIV and HIV-2, which is genetically distinct from HIV-1, has been less studied than HIV-1. Curdlan

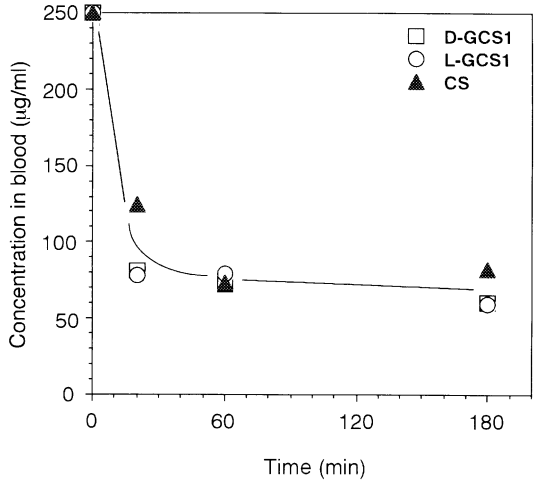


Fig. 12. Retention time of branched curdlan sulfates in plasma in vivo (rat) calculated from their APTT.

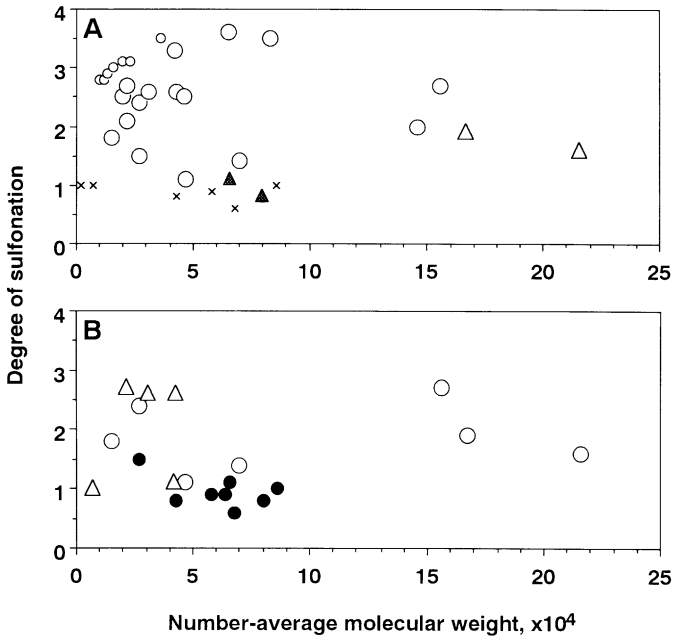


Fig. 13. Relationship between anti-AIDS virus activity of curdlan sulfates on (A) HIV-1 and (B) HIV-2 and both degree of sulfonation and molecular weight of the compounds. The inhibitory effect was evaluated by MTT method and represented by the EC_{50} concentration of less than 1.0 μg/ml (○, °, ●), 1.0–10 μg/ml (△, □), and more than 10 μg/ml (×). Symbol: °, Curdlan sulfates prepared by the CSA; ○, △, by the SPC; ●, □, ×, by the PSA methods, respectively.

sulfonate having $\bar{M}_n$ more than 10×10^4 and the DS more than 1.0 showed potent anti-HIV activity on both HIV-1 and HIV-2 strains, indicating that the structural differences seem not to be essential for anti-HIV activity on HIV-1 and -2, but the DS and molecular weights were important for high activity [133] (Fig. 13).

Ionic interactions between negatively charged polysaccharides and positively charged proteins have been analyzed in the interaction of heparin with AT-III to produce strong blood anti-coagulant activity. Considering the interactions of heparin with AT-III, sulfonated polysaccharides having negatively charged sulfonate groups might have strong interactions with positively charged helical regions in HIV envelop glycoprotein gp120, i.e. the amino acid residues of No. 506–518 in gp120 [134]. The strong inhibitory effects of curdlan sulfonate on HIV infection may be caused mainly by its absorption to gp120 to prevent the virus from binding to CD4 positive T-lymphocytes.

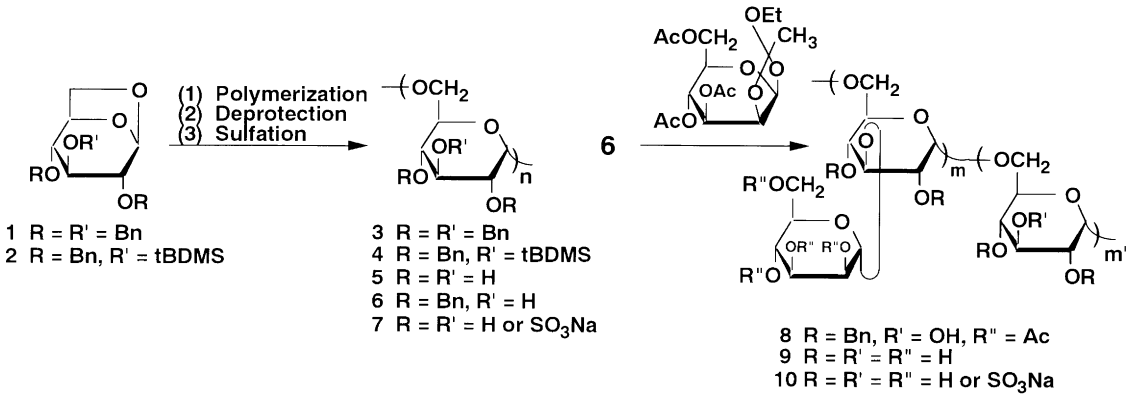
In order to analyze the interaction of curdlan sulfonate with V3 and its association with the neutralization sensitivity of HIV isolates, the effect of curdlan sulfonate on the binding of neutralizing antibodies to monomeric and oligomeric gp120 mutants in which the V3 loop was either detected or substituted by the V3 of another isolate [135]. The V3 domain represents the most probable site for the interaction with curdlan sulfonate. The V3 loop with six positively charged amino acid residues located near and at the top of the loop creates a positively charged patch which may interact and form a complex with sulfonate groups at the sixth position of carbon in each glucose unit of curdlan sulfonate.

Jeon and Uryu evaluated the interactions of sodium curdlan sulfonate with HIV by NMR methods using model compounds [136]. The model system for the interaction was constructed using curdlan

sulfonate and poly-L-lysine/HBr to simulate the α-6 helix of gp120 on HIV. The oligomeric nature of both species was characterized by ^{1}H and ^{13}C NMR. Upon mixing certain ratios of the two compounds in aqueous solution, a gel formed in the sample tube. The isolated gel was found to be composed primarily of poly-L-lysine and curdlan sulfonate, having excluded most of the two original species. Maximal gelling was attained at pH 4.0–8.4, the molecular weight $\bar{M}_n = 10 \times 10^3$ of curdlan sulfonate, and 37°C. These results suggest that curdlan sulfonate can bind strongly with poly-L-lysine via ionic interactions to produce a polyion complex. This interaction may help to explain the observed inhibition of HIV and other viruses by curdlan sulfonate. Curdlan sulfonate may bind to highly basic helical segments of virus proteins to block the binding of HIV to the T-cell and macrophage and also to induce a conformational change of gp120. As the results indicate, HIV decreases the infectivity.

6.1.2. Sulfonated dextran (dextran sulfonate)

We prepared sulfonated linear and mannose-branched dextrans and the anti-HIV and blood anti-coagulant activity was assayed [137] (Scheme 45). Dextran sulfonate is a sulfonated polysaccharide having a high negative charge, which binds strongly to thrombin and exhibits anti-coagulant activity. Since dextran sulfonate has also lipemea-clearing activity, it has been clinically used for the treatment of high blood-lipid levels in Japan. Synthetic linear and mannose-branched dextran sulfonates having a DS more than 1.0 at concentrations of 3.3 and 10 μg/ml showed complete inhibitory effect on HIV infection to the MT-4 cell in 6-day culture in vitro. No IF-positive cells were detected at these concentrations, indicating that both linear and branched dextran sulfonates had potent anti-HIV activity. The cytotoxicity was low. It was found that sulfonated mannose-branched dextran had higher anti-coagulant activity, 85 unit/mg, than that of linear dextran, 49–59 unit/mg. The highly anti-coagulant activity may be ascribed to the high DS of the branched dextran sulfonate, that is, the sulfonate group was introduced preferably into the branches to increase the DS of the polysaccharides.



Scheme 45.

6.1.3. Sulfonated ribofuranan and ribopyranan

Since the 1,4-anhydro-ribose-monomer has a high ring-opening polymerizability, we synthesized many polyribose derivatives and assayed the biological activities of sulfonated polyriboses [138]. Hatanaka reported the effect of the degree of sulfonation on the anti-HIV activity of sulfonated

Table 4
Anti-AIDS virus activity of sulfated ribofuranans and ribopyranans

No.	Sulfated ribopolysaccharide[a] (branch) (unit/mg)	S content (%)	$\bar{M}_n$[b] $\times 10^4$	EC_{50}[c] (μg/ml)	CC_{50}[d] (μg/ml)	AA[e]
1	RFSO	17.6	1.7	3.3[f]		56
2	RMFS1 (D-Mannose 13%)	16.1	1.2	3.3[f]		55
3	RDFS1 (D-Glucose 23%)	16.8	1.1	0.27	> 1000	783
4	RLFS1 (L-Glucose 29%)	16.3	0.7	2.37	> 1000	24
5	RPS1, β 100%	16.5	0.9	1.5	510	26
6	RPS2, β 100%	16.2	1.2	1.4	> 1000	36
7	PRS3, β 100%	17.9	1.2	0.1	420	n.d.
8	RPS4, β 100%	15.5	1.1	0.2	627	n.d.
9	RPS5, β 26%, a 74%	14.7	1.4	0.5	> 1000	29
10	RPS6, β 75%, a 25%	14.2	0.9	0.5	> 1000	25
11	RPDS1 (D-glucose 38%)	16.7	1.5	0.4	706	47
12	RPLS2 (L-glucose 35%)	16.8	1.1	0.3	> 1000	34
	CS[g]	14.1	7.9	0.43	> 1000	< 10
	AZT (μM)			0.0019	6.43	

[a] RFS0: sulfonated linear ribofuranan; RMFS1: sulfated ribofuranan with D-mannose branched; RDFS1: sulfated ribofuranan with D-glucose branches; RLFS1: sulfated ribofuranan with L-glucose branches. RPS: sulfonated linear ribopyranans; RPDS: sulfonated ribopyranan with D-glucose branches; RPLS: sulfonated ribopyranan with L-glucose branches.
[b] Determined by GPC.
[c] 50% Effective concentration.
[d] 50% Cytotoxic concentration.
[e] Anticoagulant activity, dextran sulfate NC-1020, 19.3 unit/mg.
[f] Minimum effective concentration.
[g] Standard curdlan sulfate.

(1 → 5)-α-D-ribofuranan. Sulfonated ribofuranan having $\bar{M}_n = 7.8 \times 10^3$ and the degree of sulfonation (DS) of 1.8 (maximum 2.0) was found to protect the infection of HIV to the MT-4 cell at concentrations as low as 10 μg/ml. However, sulfonated ribofuranans having low DS had low anti-HIV activity at any concentration. The anti-HIV activity depended remarkably on the DS. The blood anti-coagulant activity of sulfonated ribofuranans having a DS of more than 1.2 increased linearly from 10 to 30 unit/mg based on a standard dextran sulfonate (21 unit/mg) with an increase of the DS.

The results of anti-HIV activity of sulfonated ribofuranans and ribopyranans are summarized in Table 4. We synthesized branched ribofuranans and ribopyranans and then their biological activities were examined. The effects of branched and linear ribofuranans on HIV infection were evaluated by the indirect IF method or by the MTT method. Although MT-4 cells were infected and killed by HIV after a 6-day culture when less than 1 μg/ml of sulfonated ribofuranans and ribopyranans was used, 3.3 μg/ml of these sulfonates completely protected the infection [95,96]. Another type of polyribose sulfate, linear sulfonated ribopyranans had anti-HIV activity of $EC_{50} = 0.1$–1.5 μg/ml, suggesting that the linear sulfonated ribopyranans having lower molecular weights and lower DS showed lower anti-HIV activity. Sulfonated polyriboses composed of 1,4-β-pyranosidic and 1,5-α-furanosidic units exhibited high anti-HIV activity. These results suggest that the anti-HIV activity of sulfonated polysaccharides depends both on the molecular weight and DS, and not so much on the stereoregularity of synthetic polysaccharides. The

Table 5
Anti-HIV and anti-coagulant activities of sulfated octadecyl ribofuranan

No.	Sulfonated octadecyl ribofuranan			EC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)	AA[c] (unit/mg)
	$\bar{M}_n \times 10^4$	S content (%)	Degree of alkylation (%)			
1	0.6	15.7	98	0.6	> 1000	11
2	0.8	15.5	90	1.9	> 1000	13
3	0.6	15.6	88	0.6	> 1000	4
4	0.3	15.2	85	2.5	> 1000	7
5	0.7	15.6	21	13.0	> 1000	12
6	0.6	13.0	0	68.6	> 1000	14
7	0.9	14.7	0	0.6	> 1000	17
RS[d]	1.7	17.6	0	3.3[e]	> 1000	56
CS[f]	7.9	14.1	0	0.43	> 1000	

[a] 50% Effective concentration.
[b] 50% Cytotoxic concentration.
[c] Dextran sulfate H-039, 22.7 units/mg.
[d] Sulfated ribofuranan.
[e] Minimum effective concentration.
[f] Standard curdlan sulfate.

branched ribopyranans had a high anti-HIV activity of $EC_{50} = 0.3$–0.9 μg/ml, which was almost equivalent to that of curdlan sulfonate. The activity increased with an increase in the proportion of branches, probably because the introduction of branches can lead to an increase in the proportion of sulfonate groups in the polysaccharides. The branched polyriboses had a slightly higher sulfur content than that of linear ones, suggesting that the glucose branches were subjected to a higher DS than the backbone ribose units. The cytotoxicity of sulfonated ribofuranans and ribopyranans was low, $CC_{50} =$ 510 — more than 1000 μg/ml [95,96].

To know the relationship between molecular weights and anti-HIV activity, sulfonated ribofuranans having low molecular weights and having an octadecyl group at the reducing end were synthesized [139] (Fig. 14). As shown in Table 5, sulfonated ribofuranan having a low molecular weight of $\bar{M}_n = 6 \times 10^3$ and having no alkyl group (No. 6) had a low anti-HIV activity of $EC_{50} = 68.6$ μg/ml. For Nos. 1 and 3,

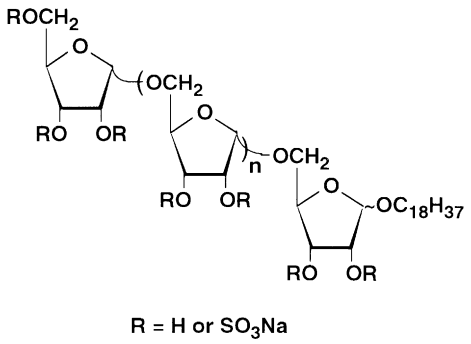


Fig. 14. Structure of sulfonated octadecyl ribofuranan.

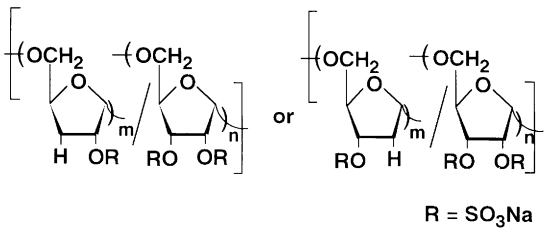


Fig. 15. Structure of sulfonated 2-deoxy or 3-deoxy ribofuranan.

sulfonated octadecyl ribofuranans having $\bar{M}_n = 6 \times 10^3$ had a high anti-HIV activity of $EC_{50} = 0.6$ μg/ml. The enhancement effect by the alkyl chain was confirmed in the case of No. 5, which has a degree of alkylation of 21 mol% and shows a low activity, $EC_{50} = 13.0$ μg/ml. On the other hand, a non-alkylated ribofuranan having $\bar{M}_n = 9 \times 10^3$ (No. 7) showed the same high activity as $EC_{50} = 0.6$ μg/ml. Therefore, the attachment of the octadecyl group to the sulfonated ribofuranan having low molecular weights caused an increase in the anti-HIV activity to the level of activity of a relatively high molecular weight sulfonated ribofuranan without the alkyl group. The anti-coagulant activity of sulfonated octadecyl ribofuranans was low, 4–13 unit/mg in comparison with that of a standard dextran

Table 6
Anti-HIV and anti-coagulant activities of sulfated deoxyribofuranan

No.	Sulfated deoxy-ribofuranan					EC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)	Anticoagulant activity[c] (unit/mg)
	Deoxy unit (mol%)	$\bar{M}_n \times 10^3$	S content (%)	$[\alpha]_D^{25}$[d] (°)	DS[e]			
2-deoxy								
1	100	5.8	10.64	+98.8	0.8	>1000	561	2
2	68	6.4	14.74	+96.6	1.3	16.6	715	2
3	16	20.1	16.53	+100.7	1.4	0.6	>1000	18
3-deoxy								
4	100	8.5	13.92	+89.3	0.9	>1000	>1000	6
5	100	7.2	14.15	+92.0	0.9	>1000	>1000	n.d.
6	46	13.5	15.40	+91.9	1.3	19.2	807	12
RS[f]		17	17.6	+83.0	1.9	3.3[g]	>1000	56
CS[h]		79[i]	14.1			0.43	>1000	< 10

[a] 50% Effective concentration.
[b] 50% Cytotoxic concentration.
[c] Dextran sulfate H-039, 22.7 units/mg.
[d] Measured in water at 25°C (*c*, 1%).
[e] Degree of sulfation per sugar unit.
[f] Sulfonated ribofuranan.
[g] Minimum effective concentration for 100% inhibition of AIDS virus infection.
[h] Standard curdlan sulfate.
[i] Weight-average molecular weight.

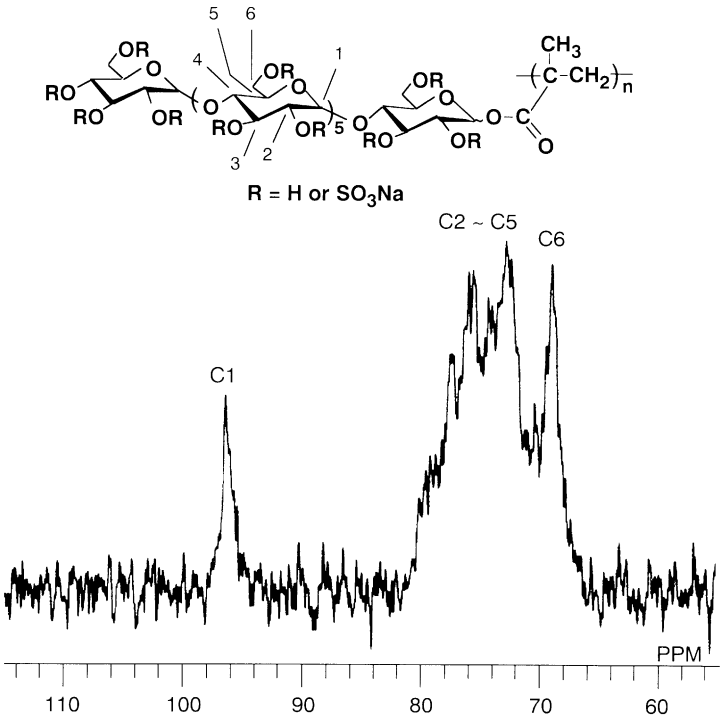


Fig. 16. ^{13}C NMR spectrum of polymethacrylate having sulfonated maltoheptaose units. $M_n = 45.3 \times 10^4$, $[\alpha]_D = +72.6°$ (c1, H_2O), DS = 2.6.

sulfonate, 22.7 unit/mg, whereas that of sulfonated ribofuranan with high molecular weight was high, 56 unit/mg.

6.1.4. Sulfonated deoxyribofuranan

In order to examine the relationship between the number of sulfonate groups and anti-HIV activity, the 1,4-anhydro-deoxyribopyranose derivative was polymerized and then sulfonated to give sulfonated polydeoxyribose. Also, copolymerization with the 1,4-anhydroribose derivative was carried out to give sulfonated ribofuranans having different degrees of sulfonation [140] (Fig. 15; Table 6). It was found that both sulfonated 2- and 3-deoxy-ribofuranans had no anti-AIDS virus activity. The anti-HIV activity increased with the decreasing ratio of the deoxy unit to give an activity as high as $EC_{50} = 0.6$ μg/ml, indicating that the number of sulfonate groups in the polysaccharide main chain was important for the high anti-HIV activity. The sulfonated deoxy-ribofuranans had a relatively low blood anti-coagulant activity of 2–10 unit/mg compared with that of sulfonated ribofuranan of 56 unit/mg. These results suggest that the anti-HIV activity strongly depended on the number of sulfonate groups in the polymer backbone.

6.1.5. Polymethacrylates with sulfonated maltoheptaose side chains

The relationship between structures and biological activities of the polymethacrylates having maltoheptaose side chains was examined [141] (Fig. 16). The methacrylates having a sulfonated

maltoheptaose side chain in every repeating unit exhibited a low anti-HIV activity within the range of 15–62 μg/ml (standard curdlan sulfonate = 0.13 μg/ml) and low anti-coagulant activity around 10 unit/mg (standard dextran sulfonate = 22.7 unit/mg). It was revealed that the anti-HIV activity increased with a decrease of the proportion of sulfonated oligosaccharide side chains. The methacrylate having 22 mol% of the oligosaccharide side chains (DS = 30) had a high anti-HIV activity in the EC_{50} = 0.3 μg/ml. The blood anti-coagulant activity also increased slightly from 9 to 18 unit/mg. These results suggest that the biological activities depended strongly on the spatial distance between sulfonated oligosaccharide substituents in the polymer main chain. The distinction and conformation of the oligosaccharide side chains also played an important role.

6.1.6. Sulfonated amino-polysaccharide

It was reported previously that the sulfonation of synthetic 1,6-α-linked 3-amino-3-deoxy-D-glucopyranan and its copolysaccharides was carried out to give dextran-type heparinoids having a sulfamide group at the C3 position of the sugar unit. The heparinoids with different sulfamide contents indicated that the anti-coagulant activity was independent of the sulfamide content, while an increase in sulfamide content lowered the toxicity [142]. Recently, we synthesized sulfonated 3-amino-3-deoxy-(1 → 6)-α-D-allopyranan and its copolysaccharides consisting of allose and glucose as the monomeric units, respectively, to elucidate the relationship between structure and biological activities such as anti-HIV and anti-coagulant activities [143] (Fig. 17). The sulfonated 3-amino-allopyranans showed potent anti-HIV activity, as manifested by the EC_{50} of 0.1 and 0.2 μg/ml and had low cytotoxicity (CC_{50} > 1000 μg/ml). The sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and glucose units also had high anti-HIV activities of EC_{50} = 0.2–0.5 mg/ml and low cytotoxicity. In contrast, the sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and allose units exhibited a somewhat lower activity (EC_{50} = 0.8–0.9 μg/ml) and a somewhat high cytotoxicity (CC_{50} = 740–797 μg/ml). The reason for this slight increase in cytotoxicity might be the presence of allose in a structure also containing sulfamide-substituted allose. The anti-coagulant activity increased with an increase in the proportion of sulfamide groups in the polysaccharide chains, as shown in Nos. 3–5 and 6–9 of Table 7. Although the sulfonated 3-amino-3-deoxyallo-pyranans (Nos. 1 and 2) had a high anti-coagulant activity of 44 and 58 unit/mg, the sulfonated copolysaccharide consisting of 3-amino-3-deoxyallose and allose units in the mole ratio of 78:22 also had a high anti-coagulant activity of 56 unit/mg (No. 3). The blood anti-coagulant activity of the sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and allose units decreased with a decrease in the proportion of the 3-amino-3-deoxyallose unit from 56 unit/mg to

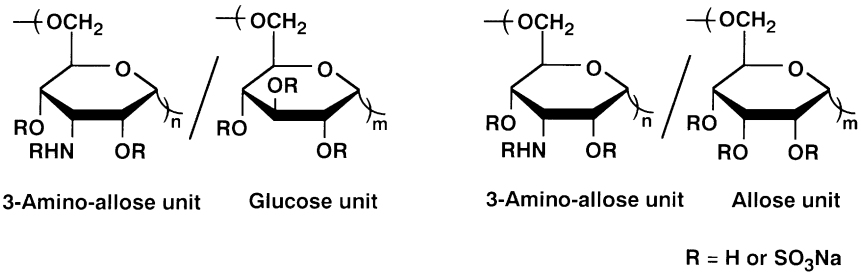


Fig. 17. Structure of sulfonated copolysaccharides consisting of 3-amino-3-deoxy-(1 → 6)-α-D-allopyranose and (1 → 6)-α-D-glucose units and 3-amino-3-deoxy-(1 → 6)-α-D-allopyranose and (1 → 6)-α-D-allose units.

Table 7
Anti-HIV and anti-coagulant activities of sulfonated 3-amino-3-deoxy-(1 → 6)-α-D-allopyranans

No.	Mole fraction of sugar unit (mol%)			$\bar{M}_n \times 10^3$	S content (%)	DS	EC_{50}[a] (μg/ml)	CC_{50}[b] (μg/ml)	Anticoagulant activity[c] (unit/mg)
	3-amino-allose	Allose	Glucose						
1	100	0	0	8.8	15.28	2.2	0.1	>1000	44
2	100	0	0	11.1	15.64	2.2	0.2	>1000	58
3	78	22	0	13.0	14.07	2.1	0.8	740	56
4	51	49	0	11.0	14.63	2.1	0.9	743	50
5	24	76	0	13.0	15.20	2.2	0.9	797	42
6	57	0	43	8.6	14.97	2.2	0.2	>1000	35
7	37	0	63	9.0	14.12	2.1	0.3	>1000	31
8	16	0	84	9.1	14.44	2.1	0.5	>1000	30
9	0	0	100	14.6	14.32	2.1	0.5	>1000	27
Dextran sulfate (H-039)				8.5	18.4	2.7	0.84	>1000	22.7
Curdlan sulfate				79.0	14.1	2.1	0.13	>1000	< 10

[a] 50% Effective concentration.
[b] 50% Cytotoxic concentration.
[c] Standard dextran sulfate H-039, 22.7 unit/mg.

42 unit/mg. In contrast, the sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and glucose units showed only moderate anti-coagulant activity of 30–35 unit/mg (Nos. 6–8). These detailed results indicate some interesting conclusions. The sulfamide group plays an important role in the interactions of the sulfamide and sulfonate group-containing copolysaccharides with blood anti-coagulant factors. The anti-coagulant activity increases which a random distribution of 3-amino-3-deoxyallose units in the amino-copolysaccharide chain. The configuration of the C3 sulfonate group is important for high anti-coagulant activity, that is, the *allo* configuration is more effective than the *gluco* one.

The anti-HIV activity of sulfonated acetamide pentosans such as xylofuranan and ribofuranan was reported [144]. 3-Acetamide-3-deoxy-2-sulfonate-(1 → 5)-α-D-xylo and -ribofuranans having the DS of 0.72 and 0.78, respectively, had a low anti-HIV activity of $EC_{50} = 21$ and 63 μg/ml. However, sulfonated copolysaccharides had high anti-HIV activity. Sulfonated copolypentosans composed of sulfonated 3-acetamide-3-deoxy-(1 → 5)-α-D-xylofuranan and ribofuranan having a degree of acetylation and sulfonation of 0.58 and 1.03, and sulfonated 3-acetamide-3-deoxy-(1 → 5)-α-D-xylofuranan and xylofuranan having the degree of acetylation and sulfonation of 0.49 and 1.20, respectively, showed a high anti-HIV activity of $EC_{50} = 0.42$ and 0.59 μg/ml. In this case, the acetamido group did not contribute to increasing the anti-HIV activity. The anti-HIV activity depended exclusively on the DS. These results were the same as those of sulfonated deoxyribofuranans we reported [38]. As mentioned above for the sulfonated amino allopyranans, we found that the sulfamide group plays an important role in the biological activities of sulfonated polysaccharides, that is, the anti-HIV and anti-coagulant activity increased with an increase of the content of sulfamide group in polysaccharides.

6.1.7. Wound healing activity of natural amino-polysaccharides, chitin and chitosan

Chitin is a natural abundant mucopolysaccharide and the supporting material of crustaceans and insects, and is known to consist of 2-acetamide-2-deoxy-β-D-glucopyranose through 1,4-β glycosidic linkage. Chitosan is 2-amino-2-deoxy-β-D-glycopyranose. Chitin and chitosan have attracted considerable attention as functional polysaccharides because of their high biological activities. Minami et al. reported dramatic biological effects of chitin and chitosan on wound healing in veterinary fields. The action mechanism of chitin and chitosan promotes the activation of chemotactic migration of polymorphonuclear cells and macrophages to increase the body's immune system (Fig. 18).

The cotton-type and suspension-type of chitosan were applied to bovine wound healing such as septic

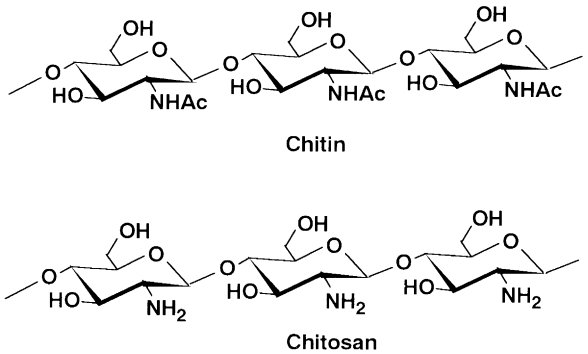


Fig. 18. Structure of chitin and chitosan.

pododermatitis including sole ulcer, interdigital phlegmon, abscess, wound, tarsal cellulitis, and arthritis in cattle. Significant effect was observed in 96% of the cases of wound healing [145].

Chitin (conventional baschitin and sponge-type) was applied to experimentally produced full skin defect (15 cm^2) in dogs [146]. The unhealed defect areas after 21 days decreased to 0.58 and 1.89 cm^2 for the baschitin and sponge-type chitin, respectively. Histologically, granulation tissues developed with neutrophil and macrophage infiltration around chitin application in both groups after 3 days. The auto-skin grafted with or without flake-type chitin was necrotized, and the unhealed defect areas at 21 days after application decreased to 1.01 and 1.75 cm^2 with and without the flake-type chitin, respectively. These results suggest that the sponge-type and flake-type chitin samples are expected to be used clinically in veterinary surgery.

Chitosan showed a biological aptitude for activating macrophages for stimulating the production of interleukin (IL-1) [147]. A large amount of chitosan (200 mg/kg) administered subcutaneously caused no physiological or hematological responses in cats, mice, and cows. However, a characteristic effect was observed in dogs, which may be induced by immunological reactions and various cytokine activations. When various amounts of chitosan (10–200 mg/kg) were administered subcutaneously to dogs, anorexia and mortality were observed in the doses above 50 and 200 mg/kg, respectively. In hematologic findings leukocytosis and increases of serum LDH2 and LDH3 isoenzymes were characteristic. From the findings of autopsy, severe hemorrhagic pneumonia was observed in all dogs. Chitosan causes lethal pneumonia to dogs [148]. Minami also reported that subcutaneous implanted chitin induced high concentrations of prostaglandin E2 [149] and cytokine productions [150].

In addition, the activation of complement component 3 (C3) by chitosan was reported. The systematic activation by subcutaneous administration of chitin, chitosan, and chitosan oligomer was investigated in dogs by determining the chemiluminescence response of circulating polymorphonuclear cells. Although chitin, chitosan oligomer, latex beads, and physiological saline did not cause the activation of PMN, chitosan caused the systemic activation of PMN in a dose-dependent manner. After 3 days of chitosan administration, the serum level of complement component 3 (C3) increased about 1.6 times that of the prechitosan C3 level. These results suggest that subcutaneous administration of chitosan induces systemic activation of both PMN and serum in dogs [151]. To elucidate the possible mechanisms of C3 activation by chitin and chitosan, the degree of deacetylation and water solubility of chitin and chitosan were investigated. For homogeneous acetylated samples, the C3 concentration decreased with an increase in the degree of acetylation. Heterogeneous acetylated samples showed C3 activation. The activation was not seen in low molecular weight samples including D-glucosamine. The important factors inducing complement activation by chitosan-based mucopolysaccharide should be solids [152].

7. Specific biological activities of natural lacquer polysaccharides

The sap of lac tree (lacquer or urushi) has been used as not only natural coating materials but also traditional medicine in Japan and China. Urushi is polymerized by an enzyme, laccase, in the presence of polysaccharides [153]. The structure of lacquer polysaccharides was characterized by sugar [154], methylation [155], and NMR analyses [156], indicating a 1,3-β-galactopyranosidic main chain having complex branches with 4-*O*-methyl glucuronic acid in the terminal. The monosaccharide components were D-galactose, 4-*O*-methyl-D-glucuronic acid, L-arabinose, and L-rhamnose. It is known that neutral

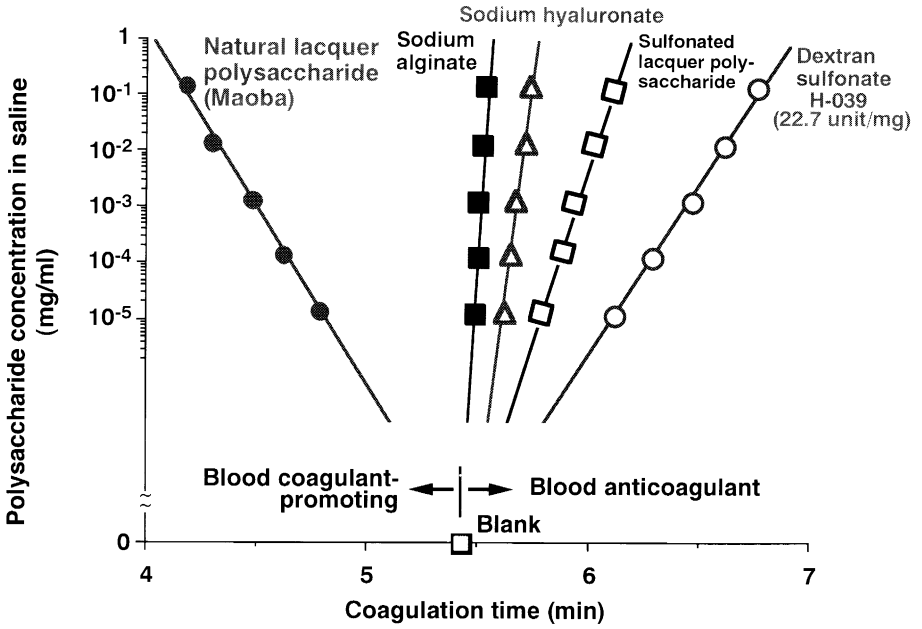


Fig. 19. Coagulation time of bovine plasma at 37°C in the presence of acidic polysaccharides. The concentration of sample solutions were made by 10 times dilutions with saline from 0.016 mg/ml to 0.016 μg/ml. Sample: 0.8 ml, bovine plasma; 1 ml, 2% $CaCl_2$; 0.2 ml. Dextran sulfonate: 0.05 ml, saline; 0.75 ml, 2% $CaCl_2$; 0.2 ml.

branched 1,3-β glucans provide a strong anti-tumor activity. We examined the biological activities such as blood coagulant, anti-tumor, anti-HIV, and anti-coagulant activities of a Chinese lacquer polysaccharide, before and after sulfonation was investigated [157].

The lacquer polysaccharide at the concentration of 0.016 mg/ml was found to shorten the coagulation time of bovine plasma by more than 1 min by comparison with that of a blank 5 min and 25 s, suggesting that the lacquer polysaccharide had a blood coagulant-promoting activity. The linear acidic polysaccharides, sodium hyarulonate and alginate, delayed the coagulation time (Fig. 19).

The lacquer polysaccharide in the dose of 50 mg/kg by an oral administration to rat reduced the weight of the Sarcoma 180 tumor by half. After sulfonation, the sulfonated lacquer polysaccharides showed no anti-tumor activity. However, it was revealed that the sulfonated lacquer polysaccharides had potent anti-HIV activity represented by the 50% protecting concentration (EC_{50}) around 0.5 μg/ml and lower blood anti-coagulant activity than that of standard dextran sulfonate. These results suggest that the lacquer polysaccharides have specific biological activities originated from the acidic branched structure and are expected to be a candidate for anti-HIV drugs as naturally occurring and reproductive resources.

8. Conclusions and future remarks

In the present paper, the author has described recent advances including his original works in the synthesis and polymerization of anhydro sugars, and the specific biological activities such as anti-HIV,

blood anti-coagulant, and anti-tumor activities of the resulting polysaccharides. In nature, there are many polysaccharides having specific biological activities. However, it is very difficult to know the structure–activity relationships, even though new instruments are being developed nowadays. Therefore, the synthetic polysaccharides having defined structures and molecular weights are very useful materials for the elucidation of the relationship. In addition, some of the artificial polysaccharides described here are expected to be new biological materials for anti-HIV and anti-tumor activities.

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References

[1] Pictet A, Sarasin V. Distillation of cellulose and starch in vacuo. Helv Chim Acta 1918;1:78–96.
[2] Pictet A. Transformation of levoglucosan into dextrin. Helv Chim Acta 1918;1:226–30.
[3] Ruckel ER, Schuerch C. Preparation of high polymers from 1,6-anhydo-2,3,4-tri-*O*-substituted β-D-glucopyranose. J Org Chem 1966;31:2233–8.
[4] Nakashima H, Kido Y, Kobayashi N, Motoki Y, Neushul M, Yamamoto N. Antiretroviral activity in a marine red alga: reverse transcriptase inhibition by an aqueous extract of *Schizymenia pacifica*. J Cancer Res Clin Oncol 1987;113:413–6.
[5] Nakashima H, Kido Y, Kobayashi N, Motoki Y, Neushul M, Yamamoto N. Purification and characterization of an avian myeloblastosis and human immunodeficiency virus reverse transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. Antimicrob Agents Chemother 1987;31:1524–8.
[6] Nakashima H, Yoshida O, Tochikura T, Yoshida T, Mimura T, Kido Y, Motoki Y, Kaneko Y, Uryu T, Yamamoto N. Sulfation of polysaccharides generates potent and selective inhibitors of human immunodeficiency virus infection and replication in vitro. Jpn J Cancer Res (Gann) 1987;78:1164–8.
[7] Uryu T. Artificial polysaccharides and their biological activities. Prog Polym Sci 1993;18:717–61.
[8] Uryu T. Polysaccharides. In: Penczek S, editor. Models of biopolymers by ring-opening polymerization. Boca Raton, FL: CRC Press, 1990. p. 133–233.
[9] Schuerch C. The chemical synthesis and properties of polysaccharides of biomediated interest. Adv Polym Sci 1972;10:173–94.
[10] Schuerch C. Synthesis and polymerization of anhydro sugars. Adv Polym Sci 1981;39:157–212.
[11] Okada M. Ring-opening polymerization of bicyclic and spiro compounds. Reactivities and polymerization mechanisms. Adv Polym Sci 1992;102:1–46.
[12] Hatanaka K. Chemical synthesis of polysaccharides. In: Dumitriu S, editor. Polysaccharides in medical applications. New York: Marcel Dekker, 1996. p. 3–20.
[13] Byrne GA, Gardiner D, Holmes FH. The pyrolysis of cellulose and the action of flame-retardants II. Further analysis and identification of products. J Appl Chem 1966;16:81–8.
[14] Caddick S. Microwave assisted organic reactions. Tetrahedron 1995;38:10,403–32.
[15] Strauss CR, Trainor RW. Developments in microwave-assisted organic chemistry. Aust J Chem 1995;48:1665–92.
[16] Allan GG, Krieger BB, Work DW. Dielectric loss microwave degradation of polymers: cellulose. J Appl Polym Sci 1980;25:1839–59.
[17] Straathof JJ, van Bekkum H, Kieboom APG. Preparation of 1,6-anhydro-glucose from 1,4-β-glucans using microwave technology. Recl Trav Chim Pays-Bas 1988;107:647–8.

[18] Raner KS, Strauss CR, Trainor RW, Trainor RW. A new microwave reactor for batchwise organic synthesis. J Org Chem 1995;60:2456–60.
[19] Miura M, Tanaka S, Ikeda K, Yokota Y, Ishibashi H, Sekiguchi I. Rapid pyrolysis of lumbers by microwave irradiation. Prog Hokkaido Br Jpn Wood Res Soc 1991;23:58–62.
[20] Miura M, Tanaka R. Refinement of levoglucosan in wood pyroligneous liquor. Mokuzai Gakkai-shi 1996;42:318–21.
[21] Micheel F, Brodde O, Reinking K. Experiments on the polycondensation of 2,3,6-tri-*O*-benzyl-D-glucopyranose and polymerization of 1,4-anhydro-β-D-glucopyranose. Justus Liebigs Ann Chem 1974:124–36.
[22] Kamitakahara H, Nakatsubo F, Murakami K. Synthesis of 1,4-anhydro-β-D-glucopyranose derivatives having acyl groups. Mokuzai Gakkai-shi 1994;40:302–7.
[23] Yoshida T, Hattori K, Choi YS, Arai M, Funaoka H, Uryu T. Synthesis and ring-opening polymerization of new 1,4-anhydro-glucopyranose derivatives. J Polym Sci A Polym Chem 1998;36:841–50.
[24] Lin JW, Schuerch C. Polymerization of 1,6-anhydro-β-D-galactofuranose. Macromolecules 1972;2:656–7.
[25] Caron S, McDonald AI, Heathcock CH. An improved synthesis of 1,6-anhydro-2,3-di-*O*-benzyl-β-D-xylo-hexopyranos-4-ulose. Carbohydr Res 1996;281:179–82.
[26] Sarkar SK, Choudhury AK, Mukhopadhyay B, Roy N. An efficient method for the synthesis of a 1,6-anhydro-β-D-galactofuranose derivative and its application in the synthesis of oligosaccharides. J Carbohydr Res 1999;18:1121–30.
[27] Yoshida T, Tsukuda T. Synthesis and polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-talopyranose. Abstr 75th Chem Soc Jpn, 1998. 25 p.
[28] Kobayashi K, Ichikawa H, Sumitomo H, Schuerch C. Sterically controlled ring-opening polymerization of a 1,6-β-D-galactopyranose derivative by neighboring group participation: (1 → 6)-β-D-galactopyranan. Macromolecules 1988;21:542–3.
[29] Ichikawa H, Kobayashi K, Sumitomo H, Schuerch C. Synthesis of a β-(1 → 6)-linked polysaccharide via ring-opening polymerization with neighboring-group participation. Carbohydr Res 1988;179:315–20.
[30] Kobayashi K, Ishii T, Okada M, Schuerch C. Steric control in ring-opening polymerization of 1,6-anhydro-galactose derivatives by neighboring group participation. Polym J 1993;25:49–57.
[31] Kobayashi K, Sumitomo H, Yasui A. Regioselectively modified stereoregular polysaccharide. 1. Polymerization of 1,6-anhydro-3-*O*-acetyl-2,4-di-*O*-benzyl-β-D-glucopyranose and synthesis of 2,4-di-*O*-benzyl-(1 → 6)-β-D-glucopyranan. Macromolecules 1979;12:1019–23.
[32] Kobayashi K, Sumitomo H. Regioselectively modified stereoregular polysaccharide. 2. Synthesis of 3-*O*-methyl-(1 → 6)-α-D-glucopyranan. Macromolecules 1981;14:250–3.
[33] Kobayashi K, Sumitomo H. Regioselectively modified stereoregular polysaccharide. 6. Synthesis of 3-deoxy-(1 → 6)-α-ribo-D-glucopyranan. Macromolecules 1983;16:710–1.
[34] Kobayashi K, Ichikawa H, Sumitomo H. Regioselectively modified stereoregular polysaccharides. 11. Synthesis of (1 → 6)-α-D-glucopyranans having one long hydrocarbon chain in position 3 in each repeating unit. Macromolecules 1990;23:3708–10.
[35] Kobayashi K, Tadano T. Synthesis of a regioselectively fluorinated polysaccharide 3-deoxy-3-fluoro-(1 → 6)-α-D-glucopyranan via ring-opening polymerization. Macromolecules 1997;30:6531–5.
[36] Uryu T, Kitano K, Ito K, Yamanouchi J, Matsuzaki K. Selective ring-opening polymerization of 1,4-anhydro-α-D-ribopyranose derivative and synthesis of stereoregular (1 → 4)-β-D-ribopyranan. Macromolecules 1981;14:1–9.
[37] Uryu T, Yamanouchi J, Kato T, Higichi S, Matsuzaki K. Selective ring-opening polymerization of di-*O*-methylated and di-*O*-benzylated 1,4-anhydro-β-D-ribopyranoses and structure proof of synthetic cellulose-type polysaccharide (1 → 4)-β-D-ribopyranan and (1 → 5)-α-D-ribofuranan. J Am Chem Soc 1983;105:6865–71.
[38] Uryu T, Yamanouchi J, Hayashi S, Tamaki H, Matsuzaki K. Selective ring-opening polymerization of 1,4-anhydro-2,3-di-*O*-benzyl-α-D-xylopyranose and synthesis of stereoregular (1 → 5)-α-D-xylofuranan. Macromolecules 1983;16:320–6.
[39] Hagino A, Yoshida S, Shinpuku T, Matsuzaki K, Uryu T. Selective ring-opening polymerization of 1,4-anhydro-α-D-lyxopyranose derivatives and synthesis of stereoregular (1 → 5)-α-D-lyxofuranan. Macromolecules 1986;19:1–7.
[40] Koyama Y, Harima K, Matsuzaki K, Uryu T. Catiojic ring-opening polymerization of 1,4-anhydro-2,3-di-*O*-benzyl-α-L-arabinopyranose and synthesis of L-arabinofuranan. J Polym Sci Polym Chem Ed 1985;23:2989–98.
[41] Uryu T, Yamanaka M, Date M, Ogawa M, Hatanaka K. Selective synthesis of polysaccharide macromonomers by ring-opening polymerization of anhydro sugar. Macromolecules 1988;21:1916–20.

[42] Yoshida T, Kida M, Uryu T. Selective ring-opening polymerization of di-*O*-tert-butyldimethylsilylated and di-*O*-*p*-bromobenzylated 1,4-anhydro-α-L-arabinopyranoses and structural analysis of free arabinans. Polym J 1987;19:923–31.
[43] Yoshida T, Arai T, Mukai Y, Uryu T. Synthesis of branched D-xylofuranan by selective ring-opening polymerization of silylated 1,5-anhydro-β-D-xylopyranose and its conversion into a blood anticoagulant. Carbohydr Res 1988;177:69–80.
[44] Kobayashi K, Schuerch C. Copolymerization of anhydroglucose and anhydromannose derivatives: structure, reactivity, and conformational analyses. J Polym Sci Polym Chem Ed 1977;15:913–26.
[45] Yoshida T, Song L, Wu C, Hatanaka K, Uryu T. Selective synthesis of cellulose-type copolymers by ring-opening copolymerization of 1,4-anhydro-α-D-ribopyranose derivatives. Chem Lett 1991:477–80.
[46] Hori MFN. Two novel synthetic methods for 1,4-anhydro-α-D-xylopyranose derivatives. Carbohydr Res 1998;309:281–6.
[47] Yoshida T, Kang B, Hattori K, Qing H, Uryu T. Ring-opening polymerization of 1,4-anhydro xylose derivative having an azido group and synthesis of stereoregular 3-amino-3-deoxy-(1 → 5)-α-D-xylofuranan. Macromolecules 1996;29:3117–22.
[48] Thiem J, Haring T. Ring-opening polymerization of *cis*-3,4-dimethoxyoxolane. Makromol Chem 1987;188:711–8.
[49] Strietholt WA, Thiem J, Howeler UFB. Synthesis and ring-opening polymerization of 1,4:2,5:3,6:-trianhydro-D-mannitol and structure studies by MNDO calculations. Makromol Chem 1991;192:317–31.
[50] Kakuchi T, Satoh T, Umeda S, Hashimoto H, Yokota K. Regio- and stereospecificity in cationic cyclopolymerization of 1,2:5,6-dianhydro-D-mannitols and synthesis of poly[(1 → 6)-2,5-anhydro-3,4-di-*O*-ethyl-D-glucitol]. Macromolecules 1995;28:4062–6.
[51] Hashimoto H, Kakuchi T, Yokota K. Synthesis of a new macromolecular ionophore with 2,5-anhydro-D-glucitol units via cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-*O*-ethyl-D-mannitol. J Org Chem 1991;56:6470–2.
[52] Kakuchi T, Satoh T, Umeda S, Hashimoto H, Yokota K. Regio- and stereoselectivity in cationic cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-methyl-D-mannitol and -L-iditol and the synthesis of poly[(1 → 6)-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol]. Macromolecules 1995;28:5643–8.
[53] Kakuchi T, Umeda S, Satoh T, Hashimoto H, Yokota K. Synthesis of poly[(1 → 6)-2,5-anhydro-D-glucitol] by cationic cyclopolymerization of 3,4-di-*O*-allyl-1,2:5,6-dianhydro-D-mannitol. Macromol Rep A 1995;32:1007–18.
[54] Satoh T, Hatakeyama T, Umeda S, Hashimoto H, Yokota K, Kakuchi T. A novel polymeric carbohydrate. Synthesis of (1 → 6)-2,5-anhydro-D-glucitol by regio- and stereoselective anionic cyclopolymerization of 1,2:5,6-dianhydro-D-mannitols. Macromolecules 1996;29:3447–52.
[55] Satoh T, Hatakeyama T, Umeda S, Hashimoto H, Yokota K, Kakuchi T. Anionic cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-methyl-L-iditol leading to (6 → 1)-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol]. Macromolecules 1996; 29:6681–4.
[56] Hatakeyama T, Kamada M, Satoh T, Yokota K, Kakuchi T. Living nature in anionic cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-methyl-D-mannitol using the potassium *tert*-butoxide/18-crown-6 initiating system. Macromolecules 1998;31:2889–93.
[57] Satoh T, Hatakeyama T, Umeda S, Yokota K, Kakuchi T. Regio- and stereoselective cyclopolymerization of 1,2:5,6-dianhydro-3,4-di-methyl-D-glucitol leading to polymers with 2,5-anhydro-3,4-di-*O*-methyl-D-mannitol and/or -L-iditol units. Polym J 1996;28:520–6.
[58] Kamada M, Satoh T, Yokota K, Kakuchi T. Regio- and stereoselective cyclopolymerization of 1,2:5,6-dianhydroallitol and 1,2:5,6-dianhydrogalactitol leading to a novel carbohydrate polymers of (2 → 6)-1,5-anhydro-D, L-galactitol. Macromolecules 1999;32:5755–9.
[59] Kamitakahara H, Nakatsubo F, Murakami K. Ring-opening polymerization of 1,4-anhydro-α-D-glucopyranose derivatives having acyl groups and synthesis of (1 → 5)-β-D-glucofuranan. Macromolecules 1994;27:5937–42.
[60] Kamitakahara H, Nakatsuo F. Substituent effect on ring-opening polymerization of regioselectively acylated 1,4-anhydro-α-D-glucopyranose derivatives. Macromolecules 1996;29:1119–22.
[61] Bochkov AF, Zaikov GE. Chemistry of the *O*-glycosidic bond. Oxford: Pergamon Press, 1979 (p. 145–8).
[62] Nakatsubo F, Kamitakahara H, Horii M. Cationic ring-opening polymerization of 3,6-di-*O*-benzyl-α-D-glucose 1,2,4-orthopivalate and the first chemical synthesis of cellulose. J Am Chem Soc 1996;118:1677–81.
[63] Kamitakahara H, Horie M, Nakatsubo F. Substituent effect on ring-opening polymerization of regioselectively acylated α-D-glucopyranose 1,2,4-orthopivalate derivatives. Macromolecules 1996;29:6126–31.
[64] Hori M, Kamitakahara H, Nakatsubo F. Substituent effect of the orthoester group on ring-opening polymerization of α-D-glucopyranose 1,2,4-orthoester derivatives. Macromolecules 1997;30:2891–6.

[65] Tsujihata S, Nakatsubo F. A novel synthetic method for α-D-galactofuranose 1,2,5-orthopivalate. Carbohydr Res 1998;308:439–43.
[66] Nishimura T, Takano T, Nakatsubo F, Murakami K. Synthetic studies of cellulose X. Selection of suitable starting materials for the convergent synthesis of cello-oligo-saccharides. Mokuzai Gakkai-shi 1993;39:40–7.
[67] Kawada T, Nakatsubo F, Umezawa T, Murakami K, Sakuno T. Synthetic studies of cellulose XII. First chemical synthesis of cellooctaose. Mokuzai Gakkai-shi 1994;40:738–43.
[68] Nishimura T, Nakatsubo F. First stepwise synthesis of cellulose analogs. Tetrahedron Lett 1996;37:9215–8.
[69] Nishimura T, Nakatsubo F. Chemical synthesis of cellulose derivatives by a convergent synthetic method and several of their properties. Cellulose 1997;4:109–30.
[70] Drauz K, Waldmann H, editors. Enzyme catalysis in organic synthesis, vols. 1 and 2. Weinheim: VCH, 1995.
[71] Kobayashi S, Shoda S, Uyama U. Enzymatic polymerization and oligomerization. Adv Polym Sci 1995;121:1–30.
[72] Kobayashi S. Enzymatic polymerization: a new method of polymer synthesis. J Polym Sci A Polym Chem 1999;37:3041–56.
[73] Kobayashi S, Kashiwa K, Kawasaki T, Shoda S. Novel method for polysaccharide synthesis using an enzyme: the first in vitro synthesis of cellulose via a nonbiosynthetic path utilizing cellulase as catalyst. J Am Chem Soc 1991;113:3079–84.
[74] Kobayashi S, Shimada J, Kashiwa K, Shoda S. Enzymatic polymerization of α-D-maltosyl fluoride utilizing α-amylase as the catalyst: a new approach for the synthesis of maltooligosaccharides. Macromolecules 1992;25:3237–41.
[75] Kobayashi S, Wen X, Shoda S. Specific preparation of artificial xylan: a new approach to polysaccharides synthesis by using cellulase as catalyst. Macromolecules 1996;29:2698–700.
[76] Kobayashi S, Kiyosada T, Shoda S. A novel method for synthesis of chitobiose via enzymatic glycosylation using a sugar oxazoline as glycosyl donor. Tetrahedron Lett 1997;38:2111–2.
[77] Kobayashi S, Kiyosada T, Shoda S. Synthesis of artificial chitin: irreversible catalytic behavior of a glycosyl hydrolase through a transition state analogue substrate. J Am Chem Soc 1996;118:13,113–4.
[78] Hicke HG, Ulbricht M, Becker M, Radosta S, Heyer AG. Novel enzyme-membrane reactor for polysaccharide synthesis. J Membr Sci 1999;161:239–45.
[79] Nishiki M, Ousaka Y, Nishi N, Tokura S, Sakairi N. Chemical synthesis of as amylose-like polysaccharide by polymerization of partially benzylated 1-thio-β-maltooctaoside derived from gamma-cyclodextrin. Carbohydr Polym 1999;39:1–6.
[80] Sakairi N, Wang L, Kuzuhara H. Modification of cyclodextrins by insertion of a heterogeneous sugar unit into their skeletons. Synthesis of 2-amino-2-deoxy-β-cyclodextrin from α-cyclodextrin. J Chem Soc Perkin Trans 1 1995:437–43.
[81] Masura V, Schuerch C. Polymerization of a cellobiose derivative to comb-shaped oligosaccharides. Carbohydr Res 1972;15:65–72.
[82] Veruovic B, Schuerch C. The preparation of comb-shaped polysaccharides by polymerization of a maltose derivative. Carbohydr Res 1970;14:199–206.
[83] Lindenberger WH, Schuerch C. Copolymerization of 1,6-anhydroglucose and 1,6-anhydromaltose derivatives. J Polym Sci Polym Chem Ed 1973;11:1225–35.
[84] Kobayashi K, Nomura K, Okada M. Chemical synthesis of a comb-shaped branched stereoregular polysaccharide, 4-*O*-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranan. Carbohydr Res 1993;242:161–6.
[85] Yoshida T, Yasuda Y, Hattori K, Uryu T. Cationic ring-opening polymerization of new 1,6-anhydro-β-lactose derivatives. Macromol Rapid Commun 1995;16:881–90.
[86] Kanematsu Y, Yoshida T. Cationic ring-opening polymerization of 1,6-anhydro-β-D-lactose derivatives. Polym Prepr Jpn 1996;45:1429–30.
[87] Endo K, Yoshida T. Synthesis of new branched polysaccharides by cationic ring-opening polymerization of anhydro ribo-disaccharide derivative. Abstr 73th Chem Soc Jpn, 1997. 3pp.
[88] Endo K, Yoshida T. Synthesis of branched polysaccharides by ring-opening polymerization of 1,4-anhydro-ribo-oligosaccharides. Polym Prepr Jpn 1998;47:221.
[89] Ichikawa H, Kobayashi K, Sumitomo H. Synthesis of a comb-shaped branched polysaccharide via ring-opening polymerization of a reactive anhydro disaccharide derivative. Macromolecules 1990;23:1884–6.
[90] Kasuya MC, Hatanaka K. The chemical synthesis of a cyclic oligosaccharide derivative with branching. Tetrahedron Lett 1998;39:9719–22.
[91] Ito H, Schuerch C. Synthesis of α-(1 → 3)-branched dextrans by copolymerization and α-D-glucosidation. J Am Chem Soc 1979;101:5797–806.

[92] Uryu T, Yamanaka M, Henmi M, Hatanaka K, Matsuzaki K. Ring-opening polymerization of 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-D-gluco-pyranose and synthesis of α-(1 → 3)-branched dextrans. Carbohydr Res 1986;157:157–69.
[93] Hatanaka K, Hirobe T, Yoshida T, Yamanaka M, Uryu T. Synthesis and sulfation of branched dextrans. Polym J 1990;22:435–41.
[94] Yoshida T, Song L, Wu C, Hatanaka K, Uryu T. Selective synthesis of cellulose-type copolymers by ring-opening copolymerization of 1,4-anhydro-α-D-ribopyranose derivatives. Chem Lett 1991:477–80.
[95] Yoshida T, Wu C, Song L, Uryu T, Kaneko Y, Mimura T, Nakashima H, Yamamoto N. Synthesis of cellulose-type polyriboses and their branched sulfates with anti-AIDS virus activity by selective ring-opening copolymerization of 1,4-anhydro-α-D-ribopyranose derivatives. Macromolecules 1994;27:4422–8.
[96] Yoshida T, Katayama Y, Inoue S, Uryu T. Synthesis of branched ribofuranans and their sulfates with strong anti-AIDS virus activity by selective ring-opening copolymerization of 1,4-anhydro-α-D-ribopyranose derivatives. Macromolecules 1992;25:4051–7.
[97] Matsuzaki K, Yamamoto I, Sato T, Oshima R. Synthesis of water-soluble branched polysaccharides and their antitumor activity, 1. Branched polysaccharides from cellulose acetate. Makromol Chem 1985;186:449–56.
[98] Matsuzaki K, Yamamoto I, Sato T, Enomoto K, Kawamura T, Hirai H. ^{13}C NMR investigations of synthetic branched polysaccharides. Carbohydr Polym 1986;6:155–63.
[99] Matsuzaki K, Yamamoto I, Sato T, Oshima R. Synthesis of water-soluble polysaccharides and their antitumor activity, 2. Branched polysaccharides from curdlan and curdlan acetate. Makromol Chem 1986;187:317–24.
[100] Yoshida T, Yasuda Y, Uryu T, Nakashima H, Yamamoto N, Mimura T, Kaneko Y. Synthesis and in vitro inhibitory effect of L-glycosyl-branched curdlan sulfates on AIDS virus infection. Macromolecules 1994;27:6272–6.
[101] Uryu T, Hatanaka K, Matsuzaki K, Kuzuhara H. Chemical synthesis of amino-group-containing (1 → 6)-α-D-glucan derivatives by ring-opening polymerization of 1,6-anhydro-azido sugars. Macromolecules 1983;16:853–8.
[102] Uryu T, Hatanaka K, Matsuzaki K. Synthesis and stereoregular hetero polysaccharides having amino-groups by ring-opening copolymerization of 1,6-anhydro-azido-sugar derivatives. J Polym Sci Polym Chem Ed 1983;21: 2203–14.
[103] Kanno K, Kobayashi Y, Nishimura S, Kuzuhara H, Hatanaka K. Synthesis of a (1 → 6)-β-linked *N*-acetyl-D-glucosamine oligosaccharide. J Carbohydr Chem 1995;14:481–90.
[104] Hattori K, Yoshida T, Uryu T. Ring-opening polymerization and copolymerization of a new benzylated 1,6-anhydro-3-azido-3-deoxy-β-D-allopyranose and synthesis of amino-polysaccharides with 1,6-α-allopyranosidic structure. Macromol Chem Phys 1997;198:29–39.
[105] Hattori K, Yoshida T, Uryu T. Ring-opening polymerization of new 1,6-anhydro-β-D-glucosamine derivatives. Carbohydr Polym 1998;36:129–35.
[106] Kang B, Hattori K, Yoshida T, Hirai M, Choi Y, Uryu T. Synthesis of 3-amino-ribofuranans having 1,5-α and -β structures by selective ring-opening polymerization of a 1,4-anhydro-3-azido-3-deoxy-α-D-ribopyranose derivative. Macromol Chem Phys 1997;198:1331–45.
[107] Borjihan G, Uryu T. Synthesis of 3-acetamido-3-deoxy-(1 → 5)-α-D-xylofuranan by ring-opening polymerization of a 1,4-anhydro-3-azido-α-D-xylopyranose derivative. Macromolecules 1998;31:5572–6.
[108] Borjihan G, Okuyama K, Katsuraya K, Uryu T. Synthesis of 3-acetamido-3-deoxy-(1 → 5)-α-D-ribofuranan by ring-opening polymerization of 1,4-anhydro-3-azido-α-D-ribopyranose derivative. Polym J 1999;31:167–71.
[109] Kobayashi K, Sumitomo H, Ina Y. Synthesis and functions of polystyrene derivatives having pendant oligosaccharides. Polym J 1985;17:567–75.
[110] Kobayashi K, Sumitomo H, Ina Y. A carbohydrate-containing synthetic polymer obtained from *N*-*p*-vinylbenzyl-D-gluconamide. Polym J 1983;15:667–71.
[111] Kobayashi K, Sumitomo H, Itoigawa T. Maltopentaose- and maltoheptaose-carrying styrene macromers and their homopolymers. Macromolecules 1987;20:906–8.
[112] Geyer U, Klemm D, Pavel K, Ritter H. Chemoenzymatic synthesis of polymerizable 11-methacryloylaminoundecanoic ester of 1- and 3-*O*-methyl-α-D-glucose in 6-*O*-position. Macromol Rapid Commun 1995;16:337–41.
[113] Yamada K, Minoda M, Miyamoto T. Controlled synthesis of amphiphilic block copolymers with pendant glucose residues by living cationic polymerization. J Polym Sci A Polym Chem 1997;35:255–61.
[114] Yamada K, Minoda M, Miyamoto T. Controlled synthesis of amphiphilic block copolymers with pendant *N*-acetyl-D-glucosamine residues by living cationic polymerization and their interaction with WGA lectin. Macromolecules 1999;32:3553–8.

[115] Yamada K, Minoda M, Miyamoto T. Controlled synthesis of glycopolymers with pendant D-glucosamine residues by living cationic polymerization. J Polym Sci A Polym Chem 1997;35:751–7.
[116] Fukudome N, Suzuki K, Yashima E, Akashi M. Synthesis of nonionic and anionic hydrogels bearing a monosaccharide residue and their properties. J Appl Polym Sci 1994;52:1759–63.
[117] Gerber P, Dutcher JD, Adams EV, Sherman JH. Protective effect of seaweed extracts for chicken embryos infected with influenza B or mumps virus. Proc Soc Exp Biol Med 1958;99:590–3.
[118] Nahmias J, Kibrick S, Bernfeld P. Effect of synthetic and biological polyanions on herpes simplex virus. Proc Soc Exp Biol Med 1964;115:993–6.
[119] Takemoto KK, Fabisch P. Inhibition of herpes virus by natural and synthetic acid polysaccharides. Proc Soc Exp Biol Med 1964;116:114–40.
[120] Ueno R, Kuno S. Dextran sulfate, a potent anti-HIV agent in vitro having synergism with zidovudine. Lancet 1987:1379.
[121] Lorentsen KJ, Hendrix CW, Collins JM, Kornhauser DM, Petty BG, Klecker RW, Flexnr C, Eckel RH. Dextran sulfonate is poorly absorbed after oral administration. Ann Internal Med 1989;111:561–6.
[122] Mitsuya H, Yarchoan R, Broder S. Molecular targets for AIDS therapy. Science 1990;249:1533–44.
[123] Morcam JA, Rosenberg RD. Regulation of the blood coagulation mechanism by anticoagulantly active heparan sulfate proteoglycans. In: Ginsberg V, Robbins PW, editors. Biology of carbohydrates, London: JAI Press, 1991. p. 47–74.
[124] Lane DA, Lindahl U, editors. Heparin. London: KABI Service, 1989.
[125] Nakashima H, Yoshida O, Tochikura T, Yoshida T, Mimura T, Kido Y, Motoki Y, Kaneko Y, Uryu T, Yamamoto N. Sulfation of polysaccharides generates potent and selective inhibitors of human immunodeficiency virus infection and replication in vitro. Jpn J Cancer Res (Gann) 1987;78:1164–8.
[126] Pauwels R, Balzarini J, Baba M, Snoeck R, Schols D, Herdewijn P, Desmyter J, DeCercq E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J Virol Meth 1988;20:309–21.
[127] US Pharmacopoeia National Formulary. USP XXI, 1985. p. 480–3.
[128] Yoshida T, Hatanaka K, Uryu T, Kaneko Y, Suzuki E, Miyano H, Mimura T, Yoshida O, Yamamoto N. Synthesis and structural analysis of curdlan sulfonate with a potent inhibitory effect in vitro of AIDS virus infection. Macromolecules 1990;23:3717–21.
[129] Kaneko Y, Yoshida O, Nakagawa R, Yoshida T, Date M, Ogihara S, Shioya S, Matsuzawa Y, Nagashima N, Irie Y, Mimura T, Shinkai H, Yasuda N, Matsuzaki K, Uryu T, Yamamoto N. Inhibitory of HIV-1 infectivity with curdlan sulfate in vitro. Biochem Pharmacol 1990;39:797–9.
[130] Gordon M, Guralnik M, Kaneko Y, Mimura T, Baker M, Lang T. A phase I study of curdlan sulfonate an HIV inhibitor. Tolerance, pharmacokinetics and effects on coagulation and on CD4 lymphocytes. J Med 1994;25:163–79.
[131] O'Brien SJ, Dean M. In search of AIDS-resistance genes. Sci Am 1995:28–35.
[132] Uryu T, Kaneko Y, Yoshida T, Mihara R, Shoji T, Katsuraya K, Nakashima H, Yamamoto N. Anti-HIV active polysaccharides and sulfated oligosaccharides. In: Yalpani M, editor. Carbohydrate and carbohydrate polymers. New York: ATL Press, 1993. p. 101–15.
[133] Yoshida T, Yasuda Y, Mimura T, Kaneko Y, Nakashima H, Yamamoto N, Uryu T. Synthesis of curdlan sulfates having inhibitory effects in vitro against AIDS viruses HIV-1 and HIV-2. Carbohydr Res 1996;276:425–36.
[134] Uryu T, Ikushima N, Katsuraya K, Shoji T, Takahashi N, Yoshida T, Kanno K, Murakami T, Nakashima H, Yamamoto N. Sulfated alkyl oligosaccharides with potent inhibitory effects on human immunodeficiency virus infection. Biochem Pharmacol 1992;43:2385–92.
[135] Jagodzinski PP, Wustner J, Kmieciak D, Wasik TJ, Fertala A, Sieron AL, Takahashi M, Tsiji T, Mimura T, Fung MS, Gorny MK, Kloczewiak M, Kaneko Y, Koznor D. Role of the V2, V3, and CD4-binding domains of gp120 in curdlan sulfonate neutralization sensitivity of HIV-1 during infection of T lymphocytes. Virology 1996;226:217–27.
[136] Jeon K, Katsuraya K, Kaneko Y, Mimura T, Uryu T. Studies on interaction mechanism of sulfonated polysaccharides as an AIDS drug by NMR. Macromolecules 1997;30:1997–2001.
[137] Yoshida T, Nakashima H, Yamamoto N, Uryu T. Anti-AIDS virus activity in vitro of dextran sulfates obtained by sulfation of synthetic and natural dextrans. Polym J 1993;25:1069–77.
[138] Hatanaka K, Nakajima I, Yoshida T, Uryu T, Yoshida O, Yamamoto N. Effect of degree of sulfonation on anti-HIV activity of synthetic (1 → 5)-α-D-ribofuranan sulfate. J Carbohydr Chem 1991;10:681–90.
[139] Choi Y, Yoshida T, Mimura T, Kaneko Y, Nakashima H, Yamamoto N, Uryu T. Synthesis of sulfated octadecyl ribooligosaccharides with potent anti-AIDS virus activity by ring-opening polymerization of a 1,4-anhydroribose derivative. Carbohydr Res 1996;282:113–23.

[140] Choi Y, Kang B, Lu R, Osawa M, Hattori K, Yoshida T, Mimura T, Kaneko Y, Nakashima H, Yamamoto N, Uryu T. Synthesis of sulfated deoxy-ribofuranans having selective anti-AIDS virus activity by ring-opening copolymerization of 1,4-anhydro ribose derivatives. Polym J 1997;29:374–9.
[141] Yoshida T, Akasaka T, Choi Y, Hattori K, Yu B, Mimura T, Kaneko Y, Nakashima H, Aragaki E, Premanathan M, Yamamoto N, Uryu T. Synthesis of polymethacrylate derivatives having sulfated maltoheptaose side-chains with anti-HIV activities. J Polym Sci A Polym Chem 1999;37:789–800.
[142] Hatanaka K, Yoshida T, Miyahara S, Sato T, Ono F, Uryu T, Kuzuhara H. Synthesis of new heparinoids with high anticoagulant activity. J Med Chem 1987;30:810–4.
[143] Hattori K, Yoshida T, Nakashima H, Premanathan M, Aragaki R, Mimura T, Kaneko Y, Yamamoto N, Uryu T. Synthesis of sulfonated amino-polysaccharides having anti-HIV and blood anticoagulant activities. Carbohydr Res 1998;312:1–8.
[144] Borjihan G, Katsuraya K, Nakashima H, Uryu T. Synthesis and anti-HIV activity of sulfated polysaccharides containing acetamido groups. Sen'i Gakkai-shi 1999;55:323–30.
[145] Okamoto Y, Minami S, Matsuhashi A, Hamada K, Yanagiya G, Ohira J, Fukumoto Y, Murakami C. Effects of chitosan on bovine wound healing. J Jpn Vet Med Assoc 1996;49:22–4.
[146] Nakade T, Uchida Y, Otomo K, Taniyama H, Okamoto Y, Matsuhashi A, Minami S. Accelerated effect of a natural polysaccharide, β-chitin on wound healing in dogs. J Jpn Vet Med Assoc 1996;49:249–52.
[147] Usami Y, Okamoto Y, Minami S, Matsuhashi A, Kumazawa N, Tanioka S, Shigemasa Y. Chitin and chitosan induce migration of bovine polymorphonuclear cells. J Vet Med Sci 1994;56:761–2.
[148] Minami S, Ohoka M, Okamoto Y, Miyatake K, Matsuhashi A, Shigemasa Y, Fukumoto Y. Chitosan-inducing hemorrhagic pneumonia in dogs. Carbohydr Polym 1996;29:241–6.
[149] Minami S, Okamoto Y, Matsuhashi A, Sashiwa H, Saimoto H, Shigemasa Y, Tanigawa T, Suzuki T, Tanioka S, Tanaka Y. Polymeric *N*-acetyl-D-glucosamine (chitin) induces prostaglandin E2 in dogs. J Vet Med Sci 1995;57:377–8.
[150] Tanigawa T, Tanaka Y, Tomita K, Sashiwa H, Saimoto H, Shigemasa Y, Okamoto Y, Minami S, Matsuhashi A. Effect of chitin on the production of interleukin-1 β in human blood monocytes. Carbohydr Polym 1992;35:147–50.
[151] Minami S, Matsuda M, Suzuki H, Okamoto Y, Matsuhashi A, Kato K, Shigemasa Y. Subcutaneous injected chitosan induces systemic activation in dogs. Carbohydr Polym 1997;33:285–94.
[152] Suzuki Y, Okamoto Y, Morimoto M, Sashiwa H, Saimoto H, Tanioka S, Shigemasa Y, Minami S. Influence of physicochemical properties of chitin and chitosan on complement activation. Carbohydr Polym 2000;42:307–10.
[153] Kumanotani J. Urushi (oriental lacquer) a natural aesthetic durable and future-promising coating. Prog Org Coatings 1995;26:163–95.
[154] Oda Y, Ishida T, Honnda K. Studies on the urushi (the latex of the lacquer tree). Part 1. Comparison between the latex of the Burmese lacquer tree and the latex of the Japanese lacquer tree. Nogei Kagakukai-shi 1962;36:527–31.
[155] Oshima R, Kumanotani J. Structural studies of plant gum from sap of the lac tree, *Rhus vermicifera*. Carbohydr Res 1984;127:43–57.
[156] Lu R, Yoshida T, Uryu T. Structural analysis of polysaccharides in Chinese lacquer by NMR spectroscopy. Sen'i Gakkai-shi 1999;55:47–56.
[157] Lu R, Yoshida T, Nakashima H, Premanathan M, Aragaki R, Mimura T, Kaneko Y, Yamamoto N, Miyakoshi T, Uryu T. Specific biological activities of Chinese lacquer polysaccharides. Carbohydr Polym 2000;43:47–54.